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Mechanisms of Cocaine Abuse Toxicity

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Mechanisms of Cocaine Abuse and Toxicity

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Mechanisms of Cocaine Abuse and Toxicity

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Preface

The seriousness of cocaine use is illustrated by population statistics on this drug. The most recent National Household Survey on Drug Abuse[1] shows that, in 1985, an estimated 22 million persons in the continental United States had at least tried cocaine. Of these, approximately 12 million individuals used it within the past year and 6 million at least once in the month prior to the survey (prior month use is considered an indication of current use). "Freebasing" has become a common occurrence and is more dangerous than snorting because of the increased pharmacological effects of the drug and the resulting drug craving. The propensity toward freebasing increases with the frequency of use. Seven percent of the population that reported using cocaine only one or two times reported smoking it, whereas 57 percent of those who reported using it 100 times or more had freebased. The popularity of the drug is underscored by figures on teenage use.[2] Over half (57 percent) of the graduating high school class of 1987 had tried illicit drugs at least once; 15.2 percent used cocaine, and crack use is estimated as 1 out of every 18 seniors (5.6 percent).[2]

Although progress is being made through biobehavioral research, a full understanding of the compulsion to keep using cocaine is, as yet, not available. Cocaine produces a number of physiological effects, including psychomotor stimulation, hypertension, tachycardia, anorexia, pupillary dilation, and euphoria. These effects have been related to the two major actions of cocaine: local anesthesia and actions in the central nervous system. Such general pharmacological knowledge, although necessary for further studies, does not provide an understanding of the basic mechanisms underlying cocaine reinforcement and its abuse.

A technical review meeting on "Mechanisms of Cocaine Abuse and Toxicity," held September 21 to 23, 1987, in Rockville, MD, addressed

the main problems encountered in treating cocaine abusers, which are twofold: to reverse or treat toxic effects of drug overdose and to develop methods of dealing with the drug craving that leads to cocaine abuse. Both of these problems have been examined in their basic biomedical aspects in this review. In particular, the review contains state-of-the-art knowledge of factors that influence drug-seeking behavior in laboratory animals, including drug vulnerability, basic neurochemical mechanisms, and neuronal sites of the "reward" pathway, as well as toxicology, perinatal consequences, and cardiovascular actions. The proceedings of this conference are presented in the following chapters, concluding with a review of the research described in the conference and, especially, areas where future efforts will be both feasible and productive.

The Editors

FOOTNOTES

1. *National Household Survey on Drug Abuse: Population Estimates 1985.* DHHS Pub. No. (ADM) 87-1539. Rockville, MD: National Institute on Drug Abuse, 1987. 79 pp.

2. Johnston, L.D.; O'Malley, P.M.; and Bachman, J.G. *Illicit Drug Use, Smoking, and Drinking by America's High School Students, College Students, and Young Adults, 1975-1987.* Rockville, MD: National Institute on Drug Abuse, in preparation.

Pharmacological Effects of Cocaine Relevant to Its Abuse

Robert L. Balster

INTRODUCTION

The purpose of this paper is to provide an introduction to the proceedings of a review sponsored by the National Institute on Drug Abuse (NIDA) on the neurobiology of cocaine. This meeting was called to survey some of what we know and to identify fruitful areas for future inquiry. Armed with a better knowledge of the neurobiology of cocaine and a fuller understanding of the relationships of the cellular actions to the pharmacological properties of cocaine that are relevant to its abuse, we should be able to design better and more targeted interventions for treatment and prevention.

In this context, then, one of the goals of this paper is to give an overview of some of the pharmacological and behavioral effects of cocaine for which we seek cellular mechanisms. The general pharmacological profile of cocaine is fairly well known. It is a short-acting central sympathomimetic stimulant with effects very similar to those of amphetamines. However, unlike amphetamines, cocaine also has local anesthetic effects. One would find little disagreement with the assertion that it is the amphetaminelike effects of cocaine that are relevant to its abuse; its local anesthetic effects are responsible only for certain side effects commonly encountered with insufflation or oral use and possibly for certain aspects of cocaine toxicity. I would like to discuss these two aspects of cocaine's pharmacology and raise some questions that I hope will be worthwhile to consider.

AMPHETAMINELIKE EFFECTS OF COCAINE

Table 1 provides a list of some of the pharmacological and behavioral effects shared by cocaine and the amphetamines. The first three effects are particularly relevant for explaining the motivation to use and abuse cocaine; the other effects may be more relevant to some of the behavioral and physiological toxicity accompanying cocaine abuse.

TABLE 1. *In vivo effects shared by cocaine and amphetamines*

1.	Subjective effects in humans	5.	Stereotyped behavior
2.	Discriminative stimulus effects	6.	Increased locomotor activity
3.	Reinforcing effects	7.	Rate-dependent increases in operant behavior
4.	Sympathomimetic physiological profile	8.	Cross-tolerance
		9.	Cross-sensitization

Subjective Effects

It is not as widely appreciated as it should be that the intoxication produced by cocaine and *d*-amphetamine is very similar, even if the duration of cocaine's effects may be somewhat shorter. For example, in the study by Fischman and colleagues (1976), 16 to 32 mg of IV cocaine given to human research subjects produced a profile of subjective effects very similar to that produced by 10 mg of *d*-amphetamine. In fact, in these experienced IV cocaine abusers, *d*-amphetamine was frequently identified incorrectly as cocaine.

Discriminative Stimulus Effects

Drug discrimination studies in animals are widely believed to be measuring properties of drugs that are relevant to the production of their subjective effects in humans (Schuster and Johanson 1988). Animals can be readily trained to discriminate an active cocaine injection from a vehicle injection. Similarly, animals can be trained to discriminate amphetamine from vehicle injections. Under these

conditions, cocaine and amphetamines often substitute for one another (Woods et al. 1987). For example, pigeons (Jarbe 1984), mice (Snoddy and Tessel 1985), and rats (Colpaert et al. 1978; Woolverton and Cervo 1986; Huang and Wilson 1986) trained to discriminate *d*-amphetamine from vehicle respond as if they had received *d*-amphetamine after cocaine injections. Similarly, *d*-amphetamine substitutes for cocaine in pigeons (de la Garza and Johanson 1985), rats (Colpaert et al. 1978; Huang and Wilson 1986; Wood and Emmett-Oglesby 1986), and rhesus monkeys (de la Garza and Johanson 1983) trained to discriminate cocaine from vehicle. These data are consistent with the similarities in the subjective effects of cocaine and amphetamines reported in humans.

Reinforcing Effects

A number of the chapters in this volume will address the extensive data on the reinforcing effects of cocaine and amphetamines. Cocaine and *d*-amphetamine result in similar patterns of IV self-administration in rhesus monkeys (Deneau et al. 1969; Johanson et al. 1976). Among those working in the area of drug self-administration, it is widely believed that cocaine may be among the most effective drug reinforcers there are. Some of the evidence for this is the wide range of experimental conditions under which cocaine can be shown to function as a reinforcer (Woods et al. 1987). A demonstration of cocaine's powerful reinforcing effects was provided by a study in my laboratory by Thomas Aigner (Aigner and Balster 1978). In this study, rhesus monkeys were given a choice between IV cocaine injections and a food pellet every 15 minutes, 24 hours a day, for 8 days. All three animals tested chose cocaine on nearly every trial and even relearned within a single day to perform the necessary response to obtain cocaine after we reversed the response requirements for cocaine and food. They lost quite a lot of weight, so we terminated the experiment after 8 days to prevent more serious health effects.

It is these powerful reinforcing effects of cocaine that are most in need of being understood, since they are probably the most relevant to the compulsive abuse of cocaine and its considerable attractiveness to users. I will have more to say about what is known about the neural substrates for these effects a little later, but first I will digress to a related subject that should be of some importance in the present context.

"Crack"

One of the factors that has undoubtedly contributed to the current crisis of concern about cocaine abuse is the emergence of a new usage pattern, cocaine freebase in the form known as "crack." Crack is typically heated and the resulting vapor inhaled. This new form of cocaine abuse has raised some important research questions as summarized in table 2.

TABLE 2. *Research questions raised by the abuse of "crack"*

1. What is the physical form of cocaine available for absorption after heating?
2. Where in the tracheobronchial tree does absorption take place?
3. Does oxidation occur and are there pyrolysis products?
4. What special toxicological problems result from the inhalation route?
5. How do the effects of cocaine differ when it is inhaled?

Many factors have contributed to the popularity of crack, but paramount among these is the belief that the intoxication experienced after inhaling crack is more desirable than that after insufflation. It may be that higher doses can be self-administered by the inhalation route. On the other hand, there may be a more profound rush by the inhalation route. This rush may be as intense as that after IV use. Laboratory studies comparing the timecourse for cocaine after inhalation and IV administration confirm that the drug effects occur very rapidly after both routes (Perez-Reyes et al. 1982).

Why should a rapid onset of drug effects produce a greater euphoric response? There is evidence from animal research that the rate of cocaine administration is a very important factor in its reinforcing efficacy. In rhesus monkeys equipped with IV catheters and trained to lever-press under a fixed-interval schedule for cocaine injections, the rate of responding varied dramatically with changes in infusion rate. Figure 1 shows this relationship (Balster and Schuster 1973).

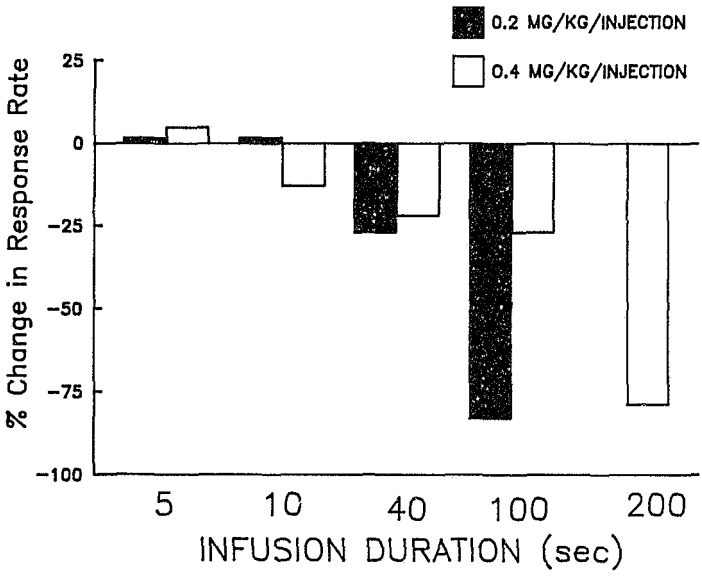

FIGURE 1. *Effects of infusion rate on the reinforcing effects of cocaine in rhesus monkeys*

NOTE: The duration of the infusion of two different doses of cocaine was varied from 5 to 200 seconds. The ordinate is the average change in response rate from the rate with a 10-second infusion duration.

SOURCE: Modified from Balster and Schuster 1973, Copyright 1973, the Society for the Experimental Analysis of Behavior, Inc.

The ordinate can be taken as a measure of the reinforcing strength of each of the conditions tested. When the rate at which the same dose of cocaine was given was decreased, i.e., when the infusion duration was increased, response rates declined. In fact, infusion rate was as important a determinant of response rate as dose. For example, the response rate when responding was maintained by 0.4 mg/kg/injection delivered over 200 seconds was similar to that maintained by 0.2 mg/kg/injection delivered in 100 seconds. Although the dose differed by twofold in these tests, the rate of cocaine delivery was the same, 0.002 mg/kg/second.

Why would the rate of cocaine infusion be such an important factor in cocaine's reinforcing effects? It could be hypothesized that, with a more rapid rate of infusion, cocaine concentrations at critical sites

of action in the brain change more rapidly, and perhaps the rate of change has important cellular consequences. At a biochemical level, rate theory of drug-receptor interactions (Goldstein et al. 1974) provides a model for considering this type of phenomenon. As a more practical matter, biochemical and pharmacological studies conducted under equilibrium or steady-state conditions cannot yield information on the second-by-second changes that occur in the brain when drugs like cocaine are taken intravenously or inhaled. Cellular approaches may need to be expanded to include more dynamic preparations capable of this temporal resolution.

BEHAVIORAL EFFECTS OF LOCAL ANESTHETICS

Cocaine, unlike the amphetamines, also has prominent local anesthetic effects; however, the most common assumption is that these local anesthetic effects have little to do with the abuse of cocaine. On the other hand, certain other local anesthetics have been shown to have behavioral effects and are capable of producing an intoxication in humans. Could the central nervous system (CNS) effects that cocaine shares with local anesthetics play a role in cocaine abuse? Could other local anesthetics have abuse potential? In the context of this conference, it may be useful to briefly review some of the behavioral pharmacology of local anesthetics.

Reinforcing Effects

Perhaps the most interesting behavioral action of certain local anesthetics is their ability to function as reinforcers in IV drug self-administration studies in monkeys. This was initially shown for procaine (Ford and Balster 1977; Hammerbeck and Mitchell 1978), but, subsequently, a number of other esteratic local anesthetics were found to have reinforcing effects as well (Johanson 1980; Woolverton and Balster 1979; Woolverton and Balster 1982). It is important to note that not all local anesthetics are reinforcers. Some, such as lidocaine and procainamide, are not.

Discriminative Stimulus Effects

A number of drug discrimination studies have compared the discriminative stimulus effects of cocaine and local anesthetics. When animals are trained to discriminate cocaine from vehicle, most drugs other than sympathomimetic stimulants do not substitute for cocaine. On the other hand, when procaine is tested, partial substitution often results (de la Garza and Johanson 1983; de la Garza and Johanson

1985; Jarbe 1984; McKenna and Ho 1980), indicating some overlap in the effects of these two drugs. Similarly, when animals are trained to discriminate procaine from vehicle, cocaine almost completely substitutes (Woolverton and Balster 1982). These data are shown in figure 2.

Tests with cocaine resulted in dose-dependent generalization from procaine; it was about tenfold more potent. Interestingly, *d*-amphetamine also substituted for procaine. If one were to assume

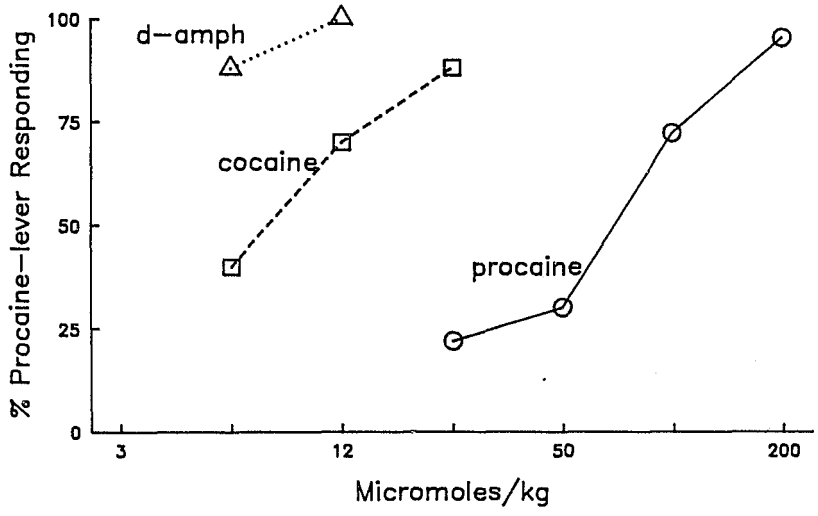

FIGURE 2. *Effects of substituting cocaine and d-amphetamine in rats trained to discriminate procaine from saline*

SOURCE: Modified from Woolverton and Balster 1982, Copyright 1982, Pergamon Journals, Ltd.

that the reason cocaine substituted for procaine was because cocaine produced local anesthetic effects similar to procaine, the results with *d*-amphetamine would be difficult to explain unless one assumed that *d*-amphetamine also had local anesthetic effects. Although this study would need to be repeated before definite conclusions could be drawn, the most parsimonious explanation at this point is that procaine, cocaine, and *d*-amphetamine share some common effects not related to local anesthesia.

Subjective Effects of Procaine

Because of the behavioral effects of procaine seen in animals, Fischman et al. (1983) compared it to cocaine after IV administration in human subjects. Although procaine resulted in a different profile of subjective effects and was considerably less efficacious than cocaine, a high dose of procaine did produce a "high," and three of the four subjects misidentified this dose of procaine as cocaine. Thus, even in human subjects there is some evidence to support the hypothesis that cocaine is not the only local anesthetic to have behavioral effects that may be related to abuse.

NEURAL BASIS OF COCAINE'S EFFECTS

The subject of this monograph is the cellular basis of cocaine's actions. Any discussion of this topic must have clearly in mind the effect for which a mechanism is sought. Cocaine has a complex pharmacology and toxicology, and different mechanisms will undoubtedly be found for different effects. In this introduction, I have focused on two aspects of the behavioral pharmacology of cocaine: its amphetaminelike effects and effects that are shared by other local anesthetics. The amphetaminelike actions of cocaine are generally well accepted and fairly well understood. On the other hand, the basis for the commonalities with local anesthetics is less clear.

The dopaminergic system undoubtedly plays an important role in most, if not all, of the amphetaminelike effects of cocaine and has also been widely implicated as a basis for its reinforcing effects (Woods et al. 1987; Kornetsky and Bain 1987). On the other hand, evidence for an exclusive role for dopamine mediation of the discriminative stimulus effects of cocaine is less clear, since selective dopamine receptor blockers are not always completely effective antagonists of the cocaine stimulus (Woods et al. 1987). It should be pointed out in this context that the behavioral pharmacology of cocaine differs considerably from selective dopamine agonists such as apomorphine and amantadine, which leads to the suspicion that many behavioral effects of cocaine, including its reinforcing and discriminative stimulus effects, may result from cocaine's interactions with a number of neurochemical systems, not just dopamine systems. The relative importance of these other biochemical effects of cocaine will be addressed in this monograph.

Another question that will be addressed is the specific mechanism for cocaine's dopaminergic actions. Unlike the amphetamines, cocaine has traditionally not been thought to release catecholamines but rather to block their reuptake, particularly that of dopamine (Heikkila et al. 1975). Yet the pharmacology of cocaine is much more similar to that of amphetamines than that of tricyclic antidepressants, perhaps due to its higher affinity for the dopamine carrier than most antidepressants. Comparison of the behavioral pharmacology of cocaine and antidepressants such as buproprion and nomifensine, which are also potent dopamine uptake blockers, should be useful in determining the role of dopamine uptake inhibition in cocaine's neuropharmacology. Nonetheless, until more is known about the pharmacological and behavioral relevance of cocaine's potent interactions with other neurotransmitter systems, it would seem unwise to focus exclusively on dopamine systems. This diversity of neurochemical actions of cocaine is a prominent feature in this monograph.

Assuming for the moment that dopaminergic effects are an important component of cocaine's behavioral effects that are relevant to its abuse, questions need to be asked about the possibility of specific dopamine pathways that may be involved and dopamine receptor subtypes that may be important. This topic, too, will be an important area of consideration in this conference.

Finally, I should comment on the problem of interpreting the data showing similarities in the effects of cocaine and local anesthetics. To put the matter most simply, either cocaine's local anesthetic effects are more important to its behavioral pharmacology than is normally believed or certain local anesthetics have some effects, possibly shared by cocaine, that account for their behavioral effects. The former option would seem unlikely. Cocaine analogs have been synthesized that allow a better separation of CNS effects and local anesthetic effects (Clarke et al. 1973; Heikkila et al. 1979), and those compounds with lower anesthetic potencies are nonetheless more potent in self-administration studies (Spealman and Kelleher 1981) and for altering schedule-controlled behavior (Spealman et al. 1977). In addition, not all local anesthetics have similar behavioral effects (Woolverton and Balster 1982), suggesting that some action of these drugs other than their local anesthetic effect may be important.

Some resolution of this question was provided by the recent data of Ritz et al. (1987), which will also be discussed in this monograph. They found that certain local anesthetics bind to the same site as cocaine, presumably on the dopamine transporter. In general, there

was a good correlation between those local anesthetics that have reinforcing effects in animal self-administration studies and those with a high affinity for this site. A similar correspondence is seen between the effects on dopamine uptake and reinforcing effects for the few cocaine analogs that have been studied in both procedures (Spealman and Kelleher 1981; Reith et al. 1986; Ritz et al. 1987). More extensive structure-activity studies of cocaine analogs and local anesthetics for biochemical and behavioral effects will be an important research direction for the future.

The fact that certain local anesthetics have affinity for the dopamine transporter and potentially inhibit dopamine uptake (Ritz et al. 1987) suggests that their behavioral effects may have a strong dopaminergic component. This is consistent with the finding that some of these drugs share discriminative stimulus effects with cocaine and amphetamine and that some serve as reinforcers. On the other hand, there are differences in the behavioral effects of local anesthetics and dopaminergic agonists. For example, there is evidence that haloperidol, which increases cocaine self-administration presumably due to antagonism of the dopaminergic effects of cocaine, fails to elicit antagonist effects in procaine self-administration (de la Garza and Johanson 1982). In our studies of procaine self-administration, we did not see overt signs of dopaminergic effects such as stereotyped behavior after the animals had received high doses (Ford and Balster 1977). What is clear from the studies of local anesthetics is that further insight into their behavioral and biochemical effects may be an important tool for understanding the neurobiology of cocaine.

CONCLUSION

In these introductory remarks, I have highlighted some of the behavioral effects of cocaine whose neural basis is the subject of this volume. Cocaine has many amphetaminelike effects but also has actions shared by certain local anesthetics. Comparisons among these diverse drugs should prove to be important in discovering the cellular basis for the actions of cocaine that are most relevant to its abuse. Although dopaminergic systems appear to play an important role, much needs to be learned about the exact nature of cocaine's interactions with this important neurotransmitter. In addition, certain aspects of the behavioral pharmacology of cocaine suggest that dopamine may not be solely responsible for cocaine's actions. Much more needs to be learned about the functional significance of cocaine's involvement with noradrenergic and serotonergic systems, and perhaps others. In addition, the cellular mechanisms for cocaine's

toxic effects may be quite different from those effects that result in its abuse. These and other related topics are the subjects of the papers that follow.

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Cocaine Receptors on Dopamine Transporters Mediate Cocaine-Reinforced Behavior

Michael J. Kuhar, Mary C. Ritz, and John Sharkey

INTRODUCTION

Cocaine is a powerful reinforcing drug that has a variety of pharmacological effects on the central nervous system. While several binding sites for ^{3}H-cocaine have been identified in the brain and in the periphery (Reith et al. 1980; Kennedy and Hanbauer 1983; Schoemaker et al. 1985; Calligaro and Eldefrawi 1987), these have not been associated with the addictive or reinforcing properties of cocaine. Recently, such a receptor for cocaine has been identified in the brain (Ritz et al. 1987a). This receptor appears to be the cocaine-binding site on the dopamine transporter on dopaminergic nerve terminals.

GOALS AND METHODS

The goal of the study was to determine the relative importance of various receptors in mediating cocaine reinforcement. To do so required a correlation between the affinities of cocaine and related drugs at the various receptor sites and the pharmacological profile of these drugs in a drug self-administration paradigm. The study included drugs that were closely related to cocaine, such as cocaine analogs, other ester-linked local anesthetics, and some other closely related psychostimulants such as methylphenidate (table 1). Other drugs, such as amphetamines, that produce similar behavioral effects were excluded because of evidence that they had different pharmacokinetic properties or functioned through somewhat different mechanisms (McMillen 1983).

It is well established that cocaine inhibits monoamine uptake (Iversen 1973; Ritz et al. 1987a), and these sites appear to be labeled by

TABLE 1. *Potencies of cocaine and related compounds in self-administration and biochemical studies*

Compound	Relative Behavioral Potency	Dopamine Uptake K_i (M)	Dopamine Uptake Relative Potency
1. WIN 35,065-2	0.26	0.26	0.41
2. WIN 35,981	0.58	0.36	0.56
3. (-)Cocaine	1	0.64	1
4. Dimethocaine	0.67	1.29	2.02
5. (+/-)Norcocaine	2.82	1.21	1.89
6. Procaine	14.1	104	164
7. Chloroprocaine	29	65	102
8. (+)Pseudococaine	33	116	183
9. Mazindol	0.17	0.023	0.036
10. Methylphenidate	1	0.39	0.61
11. (+)Amphetamine	0.20	3.6	5.63
12. Lidocaine	n.d.	3,298	5,153
13. Procainamide	n.d.	1,943	3,036
14. Diethylaminoethanol	n.d.	18,197	28,433
15. WIN 35,065-3	n.d.	385	602
16. (+)Cocaine	n.d.	136	213
17. WIN 35,428	-	0.17	0.27
18. (+/-)Cocaine	-	1.51	2.36

NOTE: The relative behavioral potencies in self-administration studies were determined by averaging values obtained from studies of cocaine reinforcement. These could not be determined (n.d.) for compounds 12 to 16 due to their low potencies or toxic side effects. Compound 17 was tested only in discrimination studies. Except in two cases, the animals utilized were monkeys. ^{3}H-mazindol was used to label the dopamine transporter (10^{-5} M nomifensine blank) in striatum. Tissues were homogenized then incubated for 1 hour in buffer (50 mM tris, 120 mM NaCl, 5 mM KCl; pH 7.8; 4 °C) containing a final ^{3}H-mazindol concentration of 4 nM. Behavioral potencies were determined as described previously (Ritz et al. 1987a).

^{3}H-cocaine (Reith et al. 1980; Kennedy and Hanbauer 1983; Schoemaker et al. 1985; Calligaro and Eldefrawi 1987). Accordingly, we decided to examine these ^{3}H-cocaine-binding sites as well as a large number of other drug-binding sites in brain to determine their

possible relationship to drug self-administration. Of the more than 25 binding sites examined, cocaine had relatively high affinity only at uptake sites for dopamine, serotonin, and norepinephrine (Ritz et al. 1987a); at sigma opiate receptors; and at muscarinic cholinergic receptors. The potency of cocaine at these sites was in the micromolar range, which is similar to the blood levels in human subjects when they experience the subjective feeling of "high" (Fischman et al. 1983; Javaid et al. 1978). Thus, these affinities were considered appropriate for a physiologically relevant cocaine receptor. Choline uptake sites and a large number of other binding sites had relatively low affinities for cocaine (Ritz et al. 1987a).

RESULTS

In general, drugs that are potent in self-administration studies are also potent inhibitors of binding at the transport sites for dopamine (table 1). Compounds that are weak in self-administration studies are correspondingly weak at the binding site. Moreover, drugs that have no detectable potency in drug reinforcement studies, despite chemical similarities (such as optical isomers), show extremely weak binding affinities.

A statistical evaluation of the data indicated that inhibition of ^{3}H-mazindol binding to the dopamine transporter was significantly related to drug self-administration of cocaine and related compounds. A multiple regression analysis relating the logarithms of the relative concentrations to inhibit binding and the logarithms of the relative behavioral doses for compounds 1 through 10 was significantly and positively associated (slope=0.72 ± 0.123, $p<.001$) (figure 1).

Additional studies using ^{3}H-GBR 12935, a more selective dopamine uptake blocker than mazindol (Van der Zee et al. 1980), gave results that were consistent with these conclusions. We have shown that ^{3}H-GBR 12935 was competitively displaced by mazindol and cocaine (Sharkey and Kuhar 1987). The affinities of cocaine and related compounds are similar at the ^{3}H-mazindol and 3H-GBR 12935 sites ($r=.96$, $p<.001$). Thus, the relative potencies of these drugs at the ^{3}H-GBR 12935 site are significantly correlated with their potencies in drug self-administration studies ($r=.92$, $p<.001$). Taken together, these findings strongly suggest that the dopamine transport mechanism is the primary mechanism associated with the reinforcing effects of cocaine.

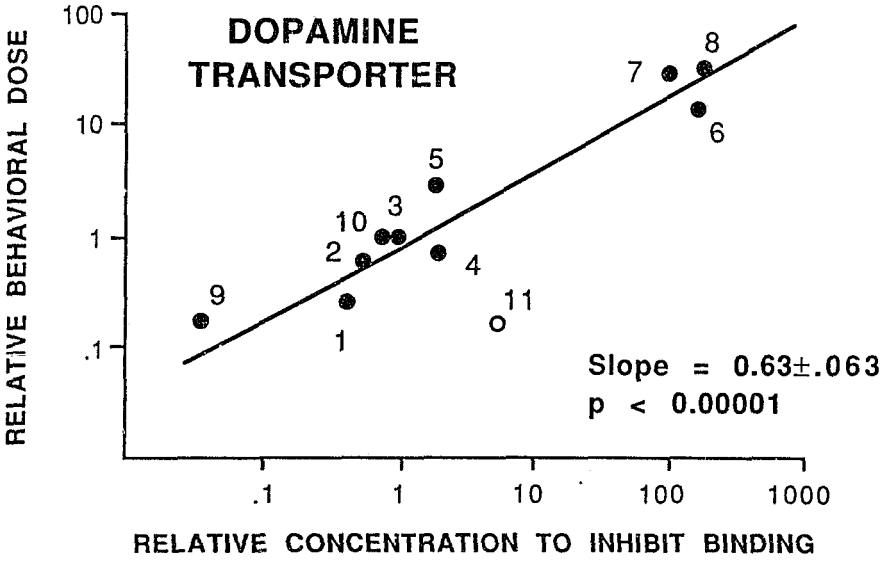

FIGURE 1. *Relation between the relative behavioral doses of cocaine and related compounds in self-administration studies and their relative inhibitory concentrations for ^{3}H-mazindol binding at the dopamine transporter*

NOTE: Linear regressions of logarithms of relative behavioral doses on logarithms of relative inhibitory concentrations for compounds 1 through 10 from table 1 were calculated using the BMDP statistical package. Value shown is slope ± *SD*. Compound 11 (open circle) was not included in the regression analysis. A two-tailed test of significance was applied ($p<.00001$).

SOURCE: Ritz et al. 1987a, Copyright 1987, American Association for the Advancement of Science.

Cocaine also has an affinity for sigma receptors in the low micromolar range (Sharkey et al., in preparation). Since sigma agonists are also reinforcing in animal models (Slifer and Balster 1983), it could be argued that cocaine's reinforcing properties could be mediated via sigma receptors. However, the relative potencies of the cocaine analogs tested at the sigma site do not correlate with their potencies in producing reinforcement in animals. For example, procaine and

lidocaine, which display only weak reinforcing properties in animal models (Woolverton and Balster 1979), are approximately equipotent with cocaine in their affinities at the sigma site (Sharkey et al., in preparation).

In our studies of the binding properties of cocaine at various receptors, we investigated the effects of cocaine at muscarinic cholinergic receptors in both brain and heart tissues (Sharkey et al., in preparation). These binding studies revealed that the (+) enantiomer of cocaine displayed an eightfold higher affinity than the (-) enantiomer. This stereospecificity is opposite to that shown by cocaine at dopamine uptake sites and in cocaine reinforcement studies. Thus, cocaine binding at muscarinic receptors does not appear to mediate its reinforcing effects. However, (-)cocaine has a K_i of about 18 M at cholinergic muscarinic receptors in the heart. Thus, these receptors would be blocked at high blood levels of cocaine that appear to be in the toxic range. Consistent with this, the average blood level of cocaine in autopsy studies where cocaine has been implicated as a cause of death was about 20 μM (Mittleman and Wetli 1984). Our data indicate that cocaine binding to ^{3}H-QNB sites is not shifted in the presence of 10 μM Gpp(NH)p, suggesting that cocaine does not act as an agonist at these sites. Thus, it is possible that inhibition of muscarinic cholinergic receptors, which would functionally enhance the sympathomimetic properties of cocaine by blocking the parasympathetic cholinergic receptors, might be involved in the cardiotoxicity of cocaine.

DISCUSSION

Many other reports are consistent with our findings relating cocaine binding to the dopamine transporter with drug self-administration. In animal studies, dopamine-containing neuronal systems, particularly in the limbic region, have been strongly implicated in the self-administration of cocaine (Wise 1984; Goeders et al. 1986; Roberts et al. 1980; Roberts and Koob 1982). Intravenous substitution of dopaminergic agonists can maintain cocaine-reinforced behavior (Woolverton et al. 1984). Self-administration of cocaine has been modulated by dopamine receptor blockers but not by drugs that affect other neurotransmitter systems. In clinical studies, it has been found that compounds that potentiate or mimic dopaminergic transmission, including methylphenidate and bromocriptine, decrease craving for cocaine (Dackis et al. 1985-1986; Khantzian et al. 1984; Gawin and Kleber 1984). By contrast, other neurotransmitter systems are apparently not involved in the mediation of the positive reinforcing

effects of cocaine. Serotonin agonists, in contrast to dopamine agonists, decrease cocaine-reinforced behavior (Mattia et al. 1986). Depletion of brain norepinephrine has little influence on self-administration of cocaine (Roberts et al. 1977). Thus, although cocaine affects norepinephrine transport and serotonin transport as well as dopamine transport, the above studies indicate that the reinforcing properties of the drug are associated with dopamine uptake inhibition and not serotonin uptake or norepinephrine uptake inhibition. While we cannot rule out the involvement of other, unknown sites in producing the reinforcing effects of cocaine, it should be emphasized that binding to the dopamine transporter accounts for 94 percent of the variance in cocaine self-administration (Ritz et al. 1987a).

At present, there is some question concerning the reinforcing properties of mazindol. Mazindol has been shown to be self-administered in animals (Wilson and Schuster 1976; Risner and Silcox 1981) and, therefore, has positive reinforcing properties. However, mazindol may have other properties, possibly even aversive properties, which could become apparent under other conditions (Chait et al. 1987). Nevertheless, the presence of other properties does not preclude the existence of quantifiable reinforcing properties of mazindol and its inclusion in our study. Also, its behavioral properties do not detract from its value as a biochemical marker for the dopamine transporter *in vitro* (Javitch et al. 1984).

We have demonstrated that ^{3}H-mazindol and ^{3}H-GBR 12935 are inhibited by cocaine and related compounds (Ritz et al. 1987b; Sharkey and Kuhar 1987). Thus, the sites recognized by ^{3}H-mazindol and ^{3}H-GBR 12935 are presumed to be the same as or very close to the site that recognizes cocaine. However, studies with ^{3}H-mazindol and ^{3}H-GBR 12935 indicate that the site labeled by cocaine, mazindol, and GBR 12935 may be distinct from the dopamine recognition site on the dopamine transporter (Javitch et al. 1984; Andersen 1987).

CONCLUSION

It has been shown that a cocaine-binding site on the dopamine transporter in brain is significantly related to the positive reinforcing properties of cocaine and some related compounds. Presumably, the main physiological effect of binding to this site is inhibition of dopamine uptake and potentiation of dopaminergic transmission. This suggestion that the dopaminergic nerve terminal is the primary site of action of cocaine is supported by the large body of evidence showing

that dopaminergic systems are affected by cocaine. In fact, intact limbic dopaminergic systems are required for the reinforcing properties of the drug. Thus, the cocaine receptor related to its dependence-producing properties is proposed to be the cocaine-binding site on the dopamine transporter.

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Cocaine Receptors on Monoamine Transporters and Sodium Channels

Maarten E.A. Reith

INTRODUCTION

Cocaine receptors can be divided into two categories.

(1) Sites that bind cocaine with high affinity and that can be detected with [^{3}H]cocaine by standard receptor-binding techniques. Two types have been characterized so far:

   (a) NA^+-sensitive receptors associated with dopaminergic nerve terminals; and

   (b) NA^+-insensitive receptors associated with serotonergic nerve terminals.

(2) Sites that bind cocaine with high affinity and that cannot be detected with [^{3}H]cocaine by standard receptor-binding techniques. The following sites are in this category:

   (a) norepinephrine uptake sites;

   (b) nonmonoaminergic sites; and

   (c) sites on sodium channels.

This chapter reviews the work done in our laboratory on the sites (1)(a), (1)(b), and (2)(c), and the implication of these sites in the effect of cocaine and cocaine analogs on locomotor behavior and stereotyped behavior in mice.

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LOCALIZATION OF COCAINE RECEPTORS: TOPOGRAPHICAL

[^{3}H]Cocaine binds with high affinity to brain membranes under conditions routinely used in most receptor-binding assays, such as the use of crude particulate fractions and 50 mM Tris-HCl at pH 7.4 to 7.7 (Reith et al. 1980a; Reith et al. 1981). Kinetic experiments indicated an extremely rapid rate of dissociation of [^{3}H]cocaine binding from mouse cerebrocortical membranes that was not related to the use of Tris-HCl buffer, which we later found to be inhibitory for cocaine binding and to act like a competitive inhibitor of cocaine binding (Reith et al. 1984a). The rate of dissociation of [^{3}H]cocaine binding, however, was the same in sodium phosphate buffer as in Tris-HCl buffer, with a half-life of approximately 25 seconds (Reith et al. 1986a).

There were distinct differences between various mouse brain regions in the effect of Na^+ on the binding of [^{3}H]cocaine (figure 1). The striatum showed pronounced Na^+-stimulated binding: a maximal fourfold increase in 25 mM Na^+. There was relatively little stimulation by Na^+ in the olfactory tubercle, another dopaminergic area. These results are similar to those reported by Kennedy and Hanbauer (1983) for Sprague-Dawley rats. Concentrations of Na^+ up to 50 mM had little or no effect in cortex and midbrain+(hypo)thalamus, slightly inhibited binding in hippocampus and pons-medulla, and decreased binding by 60 percent in the cerebellum (figure 1). Higher concentrations of Na^+ (100 to 200 mM) were inhibitory in all brain regions. Thus, Na^+-dependent binding is abundant in the striatum and present to some extent in the olfactory tubercle, and Na^+-independent binding is present in all brain regions.

The studies by Kennedy and Hanbauer (1983) indicated an association of NA^+-dependent cocaine binding in the striatum with dopaminergic nerve terminals. In contrast, the NA^+-independent binding in the cerebral cortex had the pharmacological profile of serotonin uptake sites, and was reduced by lesions caused by neurotoxins aimed at serotonergic nerve terminals (Reith et al. 1983a).

Cocaine binding observed in the striatum in the absence of NA^+ represented the NA^+-independent site of the serotonergic type observed in other brain areas as shown by the effect of 5,7-dihydroxytryptamine (Reith et al. 1985a).

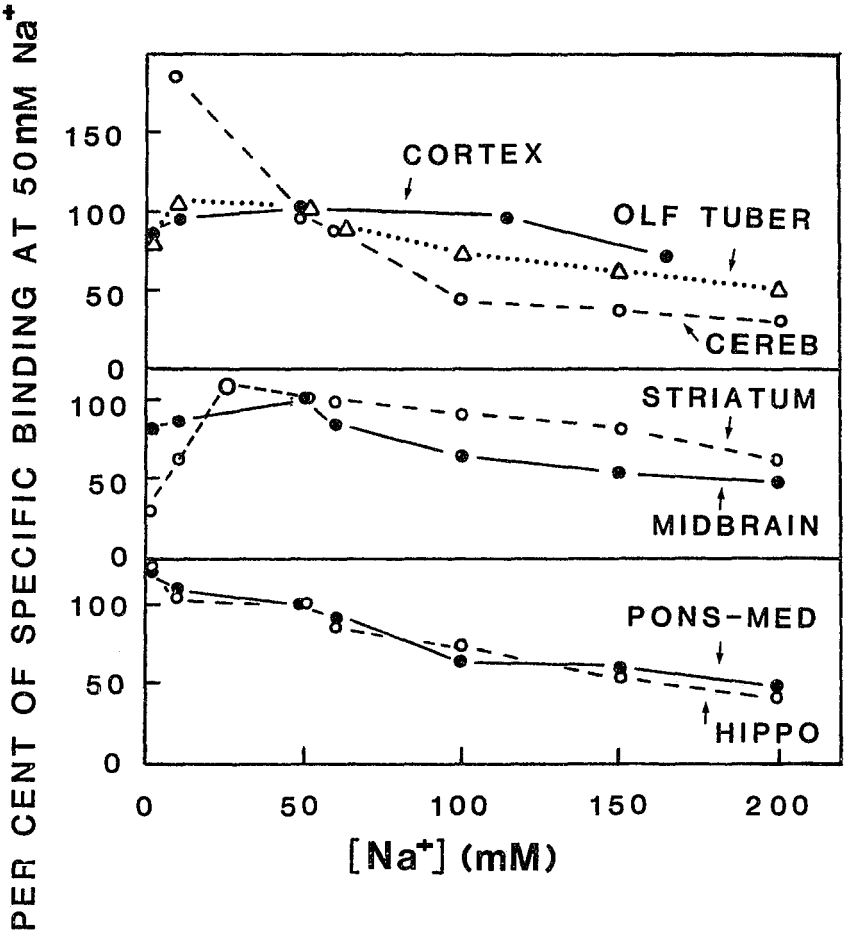


FIGURE 1. *Binding of [^{3}H]cocaine to mouse brain membranes as a function of Na^+ concentration*

NOTE: Membranes from mouse brain regions were incubated in triplicate with 16 nM [^{3}H]cocaine and varying amounts of Na^+, in the presence and absence of 30 μM cocaine for defining nonspecific binding. For the range of 0 to 50 mM Na^+, membranes were buffered with 5 mM Tris-HCl, pH 7.7; for the range of 50 to 200 mM Na^+, with 25 mM sodium phosphate buffer, pH 7.7, which contributes 48 mM Na^+ from its buffer salts. The range of 16 to 66 mM Na^+ was also studied with 25 mM HEPES buffer, pH 7.7, with similar results. The SEM in triplicate determinations was less than 7 percent. OLF TUBER, olfactory tubercle; CEREB, cerebellum; MIDBRAIN, midbrain+(hypo)thalamus; PONS-MED, pons-medulla; and HIPPO, hippocampus.

There was a significant correlation between the Na^+-independent binding of [^{3}H]cocaine at a low concentration and the neuronal uptake of [^{3}H]serotonin in various brain regions (figure 2, top left panel). Likewise, there was a good correlation between the B_{max} of NA^+-independent [^{3}H]cocaine binding and [^{3}H]serotonin uptake in the regions (figure 2, bottom left panel). There was no relationship between NA^+-independent [^{3}H]cocaine binding and uptake of [^{3}H]dopamine into noradrenergic and dopaminergic nerve terminals (figure 2, middle and right panels). There was no relationship between cocaine binding and the uptake of norepinephrine, as indicated by the lack of regional correlation (figure 2) and the ineffectiveness of lesioning by 6-hydroxydopamine in reducing the binding in the cerebral cortex (Reith et al. 1983a).

In general, the high-affinity binding of ligands to uptake sites requires the presence of sodium if sodium is required by the transport of the substrate that those sites subserve. However, there are exceptions to this rule. For instance, [^{3}H]cocaine binds with high affinity to serotonin-related sites that are Na^+-insensitive, and 8-hydroxy-2-(di-N-propylamino)tetralin binds to serotonin uptake sites in the striatum in the absence of sodium (Schoemaker and Langer 1986).

LOCALIZATION OF COCAINE RECEPTORS: SUBCELLULAR

Subcellular fractions from mouse cerebral cortex were separated to localize cocaine receptors (Whittaker 1969). The heavy and light synaptosomal mitochondrial fractions accounted for 50 percent of the recovered cocaine binding without enrichment in specific binding activity (Reith et al. 1984b). Most of the binding was recovered in the synaptosomal subfraction, although a small amount of binding in myelin had the highest relative specific activity. Since, in these experiments, Tris-HCl was used, thus reducing high-affinity binding, this localization requires cautious interpretation. However, the results are consonant with the idea that a substantial portion of cocaine binding observed in brain homogenates or crude membranes is associated with nerve terminals (Reith et al. 1984b).

INTERACTION OF COCAINE WITH THE SEROTONIN TRANSPORTER: STRUCTURE-ACTIVITY RELATIONSHIP

The following profile of cocaine congeners (table 1) was the same for Na^+-independent cocaine binding measured in synaptosomal membranes from the cerebral cortex and for [^{3}H]serotonin uptake into

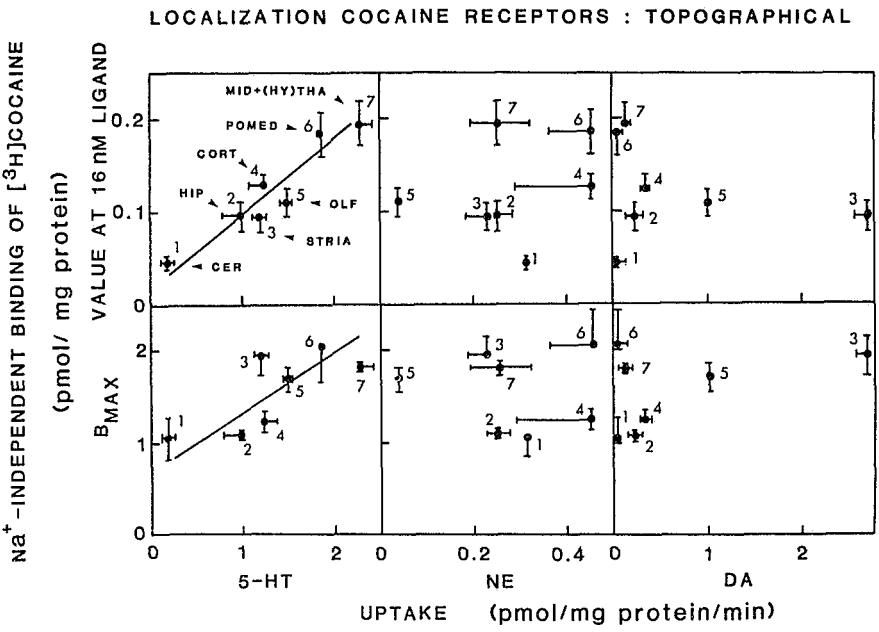


FIGURE 2. *Uptake of monoamines, and Na^+-independent binding of [^{3}H]cocaine in various mouse brain regions*

NOTE: The top panel shows Na^+-independent binding of [^{3}H]cocaine in one-point assays at 16 nM of labeled ligand; the bottom panel shows B_{max} estimates. Values are the average ± range of two independent preparations assayed in triplicate. 5-HT, neuronal uptake of [^{3}H]serotonin; NE, uptake of [^{3}H]dopamine into noradrenergic nerve terminals (defined as total uptake minus uptake in the presence of 1 μM desipramine); DA, uptake of [^{3}H]dopamine into dopaminergic terminals (estimated from the difference between uptake into both dopaminergic and noradrenergic terminals (as defined with 50 μM benztropine) and uptake into noradrenergic terminals (as defined with 1 μM desipramine)). 1, cerebellum (CER); 2, hippocampus (HIP); 3, striatum (STRIA); 4, cerebral cortex (CORT); 5, olfactory tubercle (OLF); 6, pons-medulla (POMED); and 7, midbrain+(hypo)thalamus (MID+(HY)THA).

synaptosomes from the cerebral cortex. Removal of the ester linkage between the tropane and phenyl rings of cocaine (WIN 5,428 and WIN 35,065-2) did not reduce the affinity of the molecule for the serotonin uptake sites. Moving the carbomethoxy group (R_2) on C_2 from an axial (cocaine and WIN 35,065-2) to an equatorial

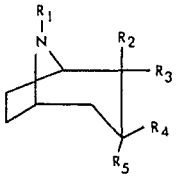
((+)-pseudococaine and WIN 35,140) position diminished the potency from fortyfold to a hundredfold. Likewise, moving the O-benzoyl group (R_4) on C_3 from an equatorial (benzoylpseudotropine) to an axial (benzoyltropine) position reduced the activity of the molecule threefold. Therefore, the position of the C_2 constituent seems to be more important than that of the C_3 constituent (Reith et al. 1986b). Removal of the C_2 constituent (benzoylpseudotropine vs. cocaine) reduced the activity tenfold, but had a less profound effect than removal of the methylester from the C_2 position of cocaine (benzoylecgonine), which rendered the molecule inactive. An isomeric pair, WIN 35,065-3(d) and WIN 35,065-2(l), showed a 500-fold stereospecificity for the l-form.

Mouse brain extracts contained material that inhibited the binding of [^{3}H]cocaine to mouse cerebrocortical membranes (Reith et al. 1980b). The material was only partially purified, and many attempts (unpublished) at increasing the specific activity of the inhibitor(s) were unsuccessful. It is not known whether the inhibitory material that we studied is specific toward cocaine-binding sites associated with serotonin uptake. Hanbauer and colleagues (1985) have reported the existence of endogenous inhibitors of the dopamine-related binding sites.

INTERACTION OF COCAINE WITH THE SEROTONIN TRANSPORTER: A MODEL SYSTEM

Although brain slices and synaptosomes have been used successfully for demonstration of high-affinity uptake of serotonin and the effect of cocaine, the description of the precise properties of the transporter is confounded in these preparations by the presence of storage granules, enzymes involved in the metabolism of serotonin, and Na^+, K^+-ATPase, which plays a role in the maintenance of the Na^+ gradient. A less complex preparation of plasma membrane vesicles can be obtained after osmotic shock of synaptosomes that has been proven extremely useful for the study of the transport of gamma-aminobutyric acid (Kanner 1978), glutamate (Kanner and Sharon 1978; Kanner and Marva 1982), and tryptophan (Herrero et al. 1985). Plasma membrane vesicles from blood platelets have often been used as a model system for brain serotonergic nerve terminals (Stahl and Meltzer 1978). However, recent studies have emphasized differences that exist between blood platelets and brain serotonergic nerve terminals.

TABLE 1. *Cocaine congeners used in study of structure-activity relationships*

	$R_{2(3)}/R_{3(2)}$ H/$COOCH_3$ or H	$R_{4(5)}/R_{5(4)}$ H/(OCO)-Phe or $OCOCHCH_2OHPHE$
Cocaine	Axial	Equatorial
(+)-pseudococaine	Equatorial	Equatorial
Benzoylpseudotropine		Equatorial
Benzoyltropine		Axial
Allococaine	Axial	Axial
Allopseudococaine	Equatorial	Axial
Atropine		Axial
Win 35,065-2 (l)	Axial	Equatorial
Win 35,065-3 (d)	Axial	Equatorial
Win 35,140	Equatorial	Equatorial

Therefore, in our laboratory, plasma membrane vesicles from mouse cerebrocortical synaptosomes were used as a tool to examine the serotonin transporter. Synaptosomes were isolated and osmotically shocked and resealed as described by Kanner (1978). In 10 seconds, the concentration of serotonin doubled in assays of uptake in membrane vesicles. The uptake of serotonin was saturable, with a K_m in the range of the values reported for synaptosomes (50 to 150 nM) (Ross 1982). In contrast to synaptosomes, plasma membrane vesicles have no exogenous energy source for uptake except for ion gradients. It was necessary to have an Na^+ gradient ($[Na^+]_{out}$ greater than $[Na^+]_{in}$), and uptake was stimulated by the presence of internal K^+ and by a K^+ gradient ($[K^+]_{in}$ greater than $[K^+]_{out}$). A membrane potential per se did not drive uptake. Cocaine inhibited uptake of serotonin, with an IC_{50} value of 347 nM and with a Hill number of 0.9, consonant with a mechanism of competitive inhibition. In future experiments, this model system will be used as a tool to study in more detail the relationship between the site where cocaine

interacts to inhibit uptake and the substrate recognition site for serotonin.

INTERACTION OF COCAINE WITH THE DOPAMINE TRANSPORTER: STRUCTURE-ACTIVITY RELATIONSHIP AND BEHAVIOR

The structure-activity relationship of cocaine congeners for the dopamine uptake transporter was generally the same as that for the serotonin transporter. The following were exceptions to this rule: removal of the ester linkage between the tropane and phenyl rings made the molecule more potent toward the dopamine transporter (Reith et al. 1986b); N-demethylation (to norcocaine) resulted in some loss of activity; and C_3 epimerization of benzoylpseudotropine (yielding benzoyltropine) had a greater effect in reducing the potency toward the dopamine transporter (sixfold) than toward the serotonin transporter (threefold). Such differences may indicate that cocaine and cocaine congeners interact at different sites on the dopamine and serotonin transporters, or that there are different environments around the cocaine-binding sites on the two types of transporters (Reith et al. 1986b). The correlation we observed between the potencies of cocaine congeners in inhibiting Na^+-dependent cocaine binding and in inducing stereotyped sniffing (Reith et al. 1986b) is in agreement with the notion that the Na^+-dependent cocaine-binding sites are involved in cocaine-induced increases in stereotyped behavior; this, in turn, fits in with the hypothesis that striatal dopamine is involved in the generation of such behavior (Beninger 1983). It should be emphasized here that it was necessary to administer the cocaine congeners intracerebroventricularly in order to observe the stereotyped behavior, because intraperitoneal administration of most cocaine congeners exhibited a depressant rather than a central stimulatory effect on behavior (see below).

RELATIONSHIP OF THE CHARACTERISTICS OF COCAINE BINDING TO ITS EFFECTS ON MONOAMINE UPTAKE

If an inhibitor competes for binding or uptake by a competitive mechanism, its IC_{50} can be converted to an inhibition constant (Cheng and Prusoff 1973). However, caution should be exercised in comparing the absolute values of potencies of compounds in uptake and binding assays. Conditions under which the assays are carried out should be considered. For instance, Tris-HCl buffer inhibits [^{3}H]cocaine binding and shifts the potencies of drugs toward higher values (Reith et al. 1986b), and brain slices generally give higher IC_{50} values for inhibitors of monoamine uptake than synaptosomal

preparations (Ross and Renyi 1975). Thus, in the striatum, there is good correspondence between the absolute value of drugs in inhibiting [^{3}H]cocaine binding to membranes and in inhibiting dopamine uptake into the slices (Kennedy and Hanbauer 1983), while an example of lack of correspondence is the binding of [^{3}H]imipramine to serotonin uptake sites in brain, which has a binding affinity an order of magnitude smaller (Langer et al. 1980; Reith et al. 1983b) than the inhibition constant of imipramine in inhibiting [^{3}H]serotonin uptake into brain synaptosomes (Koe 1976). We have shown that this discrepancy is probably caused by the different temperatures at which the two assays are routinely carried out: 0 °C for imipramine binding and 37 °C for serotonin uptake (Reith et al. 1984c).

In the absence of Tris-HCl and presence of 50 mM Na^+, there is a good agreement between the absolute values for cocaine and cocaine congeners in inhibiting [^{3}H]cocaine binding to cerebrocortical membranes and the values in inhibiting [^{3}H]serotonin uptake into cerebrocortical synaptosomes; there is also a good agreement between the inhibition of [^{3}H]cocaine binding to striatal membranes and that of [^{3}H]dopamine uptake into striatal synaptosomes (Reith et al. 1986b). These results are consonant with the suggestion that cocaine analogs are competitive inhibitors of monoamine uptake and are not translocated themselves by the uptake carrier. In contrast, substrates such as serotonin or dopamine can require higher levels in inhibiting the binding of a ligand to the uptake carrier than for inhibition in the uptake assay because transport is a multistep reaction whereas binding is not. More serotonin and dopamine are required to inhibit [^{3}H]cocaine binding than to inhibit monoamine uptake into synaptosomes (Reith et al. 1986b). It is not necessary to invoke an allosteric linkage between the binding sites and the substrate recognition sites for uptake. Attempts at demonstrating an allosteric relationship have not been successful. For instance, there is little or no effect of the substrates serotonin and dopamine on the dissociation rates of [^{3}H]cocaine binding (Reith et al. 1986a). Drugs that have releasing effects in addition to uptake inhibitory effects, such as amphetamine, may produce an IC_{50} in inhibiting uptake that is lower than the true value because of the dopamine-releasing effect of amphetamine.

INTERACTION OF COCAINE WITH THE DOPAMINE TRANSPORTER: THE EFFECT OF LONG-LASTING BLOCKADE BY METAPHIT

Metaphit (1-(1-(3-isothiocyanathophenyl)-cyclohexyl)piperidine), an analog of phencyclidine (PCP), has been proposed to specifically

acylate PCP receptors and not opioid, muscarinic, or benzodiazepine receptors (Rafferty et al. 1985). Since PCP has appreciable affinity for the dopamine uptake sites (Kennedy and Hanbauer 1983; Schoemaker et al. 1985), we examined the possibility of using metaphit as a long-acting blocker of dopamine uptake sites for the antagonism of effects of cocaine mediated by the dopamine transporter. Treatment of striatal membranes with metaphit produced a concentration-dependent loss of [^{3}H]cocaine binding, due to an effect on maximal binding rather than to the affinity (Berger et al. 1986). Since the membranes were washed three times after metaphit treatment, these results suggest that metaphit binds irreversibly to, or dissociates slowly from, the Na^+-dependent cocaine-binding sites. Similar results have been reported recently by Schweri et al. (1987) for the binding of [^{3}H]methylphenidate to rat striatal membranes. The behavioral effects of PCP in inducing stereotypy and ataxia are antagonized by pretreating rats intracerebroventricularly with metaphit 24 hours prior to PCP administration (Contreras et al. 1985), and the density of PCP receptors is reduced by pretreating animals 24 hours prior with metaphit both intracerebroventricularly and intravenously (Contreras et al. 1986). Pretreatment of mice with metaphit (20 mg/kg intravenously) antagonizes the locomotor stimulation caused by various psychostimulant drugs tested 24 hours later (Sershen et al. 1988). Metaphit antagonizes drugs that exert their hypermotive effect primarily by inhibition of the uptake of dopamine, such as methylphenidate, mazindol, cocaine, and GBR 12909. Subsequent experiments did not demonstrate a blockade of the dopamine transporter at the time of the behavioral testing. First, pretreatment with metaphit did not reduce the *in vivo* labeling of the dopamine transporter with [^{3}H]GBR 12935. Second, pretreatment with metaphit did not antagonize the locomotor stimulation evoked by amphetamine, in spite of the fact that the effect of amphetamine involves the release of dopamine by the same carrier that translocates dopamine into the terminal. Third, *ex vivo* experiments did not indicate any difference between metaphit- and saline-pretreated animals in the uptake of dopamine into synaptosomes or slices (Sershen et al. 1988).

The possibility was considered that metaphit affects the locomotor responses to stimulant drugs by a mechanism unrelated to uptake blockade. A study of levels of monoamines and their metabolites in various brain regions showed a consistent increase in homovanillic acid (HVA) in all regions studied--striatum, olfactory tubercle, and cerebral cortex--24 hours after pretreatment with metaphit (Sershen et al. 1988). Metaphit had no effect on the disappearance rate of

3,4-dihydroxyphenylacetic acid and HVA from the striatum during inhibition of monoamine oxidase with pargyline. If the increase in HVA reflects a greater rate of dopamine catabolism in metaphit-pretreated animals, it could explain the lack of locomotor stimulation of blockers of dopamine uptake in these animals, resulting from a rapid breakdown of extracellular accumulated dopamine.

INTERACTION OF COCAINE WITH SODIUM CHANNELS: STRUCTURE-ACTIVITY RELATIONSHIP AND BEHAVIOR

As do other local anesthetic drugs, cocaine can be expected to interfere with the uptake of sodium through voltage-regulated sodium channels. For studying the interaction of cocaine and cocaine congeners with sodium channels, we used [^{3}H]batrachtochotoxinin-A20-α-benzoate (BXT-B) as a ligand for the proteins involved in the gating of the channel. It is known that local anesthetic drugs inhibit BTX-B binding to sodium channel preparations from brain, most likely by an allosteric mechanism shifting the equilibrium of the channel toward the nonconducting state (Postma and Catterall 1984; Reith et al. 1986c). We assessed the potency of cocaine congeners by two methods that gave the same result: (1) determination of the inhibition constant for the equilibrium binding of [^{3}H]BTX-B, and (2) estimation of the concentration that is required to enhance by a factor of two the dissociation rate of [^{3}H]BTX-B binding initiated by an excess of aconitine, an alkaloid that acts at the same site as BTX-B (Catterall 1977). The observed enhancement of the dissociation rate agrees with the suggestion that cocaine and cocaine congeners act at a site in the sodium channel that is allosterically linked with the site labeled by [^{3}H]BTX-B (Reith et al. 1986c). As found for the activity toward monoamine transporters, the ester linkage between the tropane and phenyl rings of cocaine was not necessary for local anesthetic activity; WIN 35,140 and WIN 35,004 (table 1) had the same potency as cocaine, whereas WIN 35,428 and WIN 35,065-3 were approximately threefold to fourfold less potent than cocaine. Moving the O-benzoyl group on C_3 from an equatorial (benzoylpseudotropine) to an axial (benzoyltropine) position reduced the potency threefold, and removal of the O-benzoyl group from C_3 (ecgonine methylester) had a greater effect in reducing potency (1,000-fold) than modifying the group (atropine, sixfold reduction). In contrast to the structure-activity relationships for monoamine uptake inhibition, however, N-demethylation (norcocaine) increased the inhibitory activity fourfold. Moving the carbomethoxyl group on C_2 from an axial (cocaine and WIN 35,428) to an equatorial ((+)-pseudococaine and WIN 35,140) position increased the potency by a

factor of 4; (-)-pseudococaine, which is the enantiomer of (+)-pseudococaine and has the C_2- and C_3-substituents also in the equatorial positions, was three times less potent than (+)-pseudococaine.

To our surprise, intraperitoneal administration of most cocaine congeners did not induce locomotor stimulation as cocaine itself did. Instead, spontaneous locomotor behavior was inhibited; the animals were less alert, but had the eyes open and did not assume postures normally associated with sleep (Reith et al. 1985b). There was a reasonable agreement between the rank order of potencies of cocaine congeners and other local anesthetics in depressing locomotor behavior and in inhibiting [^{3}H]BTX-B binding (Reith et al. 1986c). Such a comparison has to be judged with caution, since drugs applied *in vivo* must be transported to their presumed sites of actions, are susceptible to metabolizing enzymes, and may have multiple effects. Nevertheless, the observed correlation is consonant with an involvement of sodium channels in the inhibition of locomotor behavior by cocaine congeners. The present results do not indicate whether central sodium channels are involved; there is some evidence suggesting action at the peripheral level (Reith and Lajtha 1986). It is possible that multiple targets at the peripheral levels are involved in the effect of local anesthetics, such as the cardiovascular system, the neuromuscular junction, and the lungs (Ritchie and Greene 1980).

INTERACTION OF COCAINE WITH SODIUM CHANNELS: THE PHOSPHATIDYLINOSITOL RESPONSE

Hydrolysis of inositol phospholipids has received much recent attention as an effector system coupled to alpha$_1$-adrenergic, 5-HT$_2$-, muscarinic-cholinergic, and certain peptidergic receptors, with diacylglycerol and inositol 1,4,5-triphosphate as the second messengers (Berridge et al. 1983; Nahorski et al. 1986). In addition to agonists acting on these receptors, various agents that activate voltage-dependent sodium channels have been found to stimulate polyphosphoinositide turnover (Gusovsky et al. 1986), suggesting that sodium channel activity per se can have an input to phosphatidylinositol systems. The blockade of monoamine uptake by cocaine may lead to an enhanced polyphosphoinositide turnover in neurons innervated by norepinephrine and serotonin, while the inhibition of sodium flux by cocaine may lead to reductions in inositol phospholipid hydrolysis. Experiments were carried out in our laboratory to test the latter possibility. Production of inositol phosphates in the presence of lithium slices was taken as an index of phosphatidylinositol phosphate

hydrolysis, and [^{14}C]guanidine flux through activated sodium channels in synaptosomes served as a model for sodium flux. It was found that cocaine indeed interacts with the phosphoinositide system at concentrations that inhibit sodium flux. The stimulation of inositide hydrolysis by the sodium channel activators batrachotoxinin (BXT), aconitine, and scorpion venom in mouse cerebrocortical slices was reduced by the presence of 5 μM tetrodotoxin, a blocker of sodium channels. Cocaine and tetracaine were found to shift the dose-response curve of BTX in stimulating inositide hydrolysis to the right. In addition, cocaine and tetracaine inhibited BTX-elicited (0.05 μM) inositide hydrolysis, with IC_{50} values of approximately 100 and 2 μM, respectively. Cocaine and tetracaine also inhibited veratridine-stimulated (60 μM) [^{14}C]guanidine uptake into synaptosomes, with IC_{50} values of approximately 140 and 25 μM, respectively. These results do not negate the possibility that cocaine can lead to increased inositide hydrolysis by monoamine uptake blockade. The data, however, do show that the increase in inositide hydrolysis induced by a sodium channel activator can be counteracted by concentrations of cocaine that inhibit the flux of guanidine through activated sodium channels (Kim and Reith 1988).

REPEATED COCAINE ADMINISTRATION, TOLERANCE, AND SENSITIZATION, AND THE CONVERSION TO N-HYDROXY-NORCOCAINE

Repeated intraperitoneal injections of cocaine in rodents can produce both sensitization and tolerance to its behavioral effects (Post and Contel 1983). These results are related to the type of behavior under study, such as locomotion and stereotypy, which are usually susceptible to the sensitization phenomena. Cocaine discrimination and operant conditioning produce tolerance effects. Three other variables that have been considered in studies of a related psychostimulant drug, amphetamine, have not been addressed in cocaine studies: (1) the size of the dose, (2) the regimen of administration (intermittent vs. continuous), and (3) pharmacodynamics.

(1) The size of the dose was studied with cocaine and WIN 35,065-2, one of the few phenyltropane analogs of cocaine that, upon intraperitoneal administration, stimulate locomotor behavior of mice. Locomotor sensitization to the locomotor stimulatory effects developed during daily administration of either compound, with similarly shaped dose-response curves (Reith 1986). Dosages giving optimal sensitization were 25 mg/kg/day for cocaine and 3 mg/kg/day for WIN 35,065-2. At higher doses,

cocaine produced tolerance to stimulation on day 3, as did WIN 35,065-2 (Reith 1986). This tolerance at the higher dose did not appear to result from an increase in nonambulatory (or stereotyped) behavior. Therefore, the size of the dose of cocaine is a crucial variable in dosage schedules for studying the sensitization to its effect on locomotor behavior.

(2) Intermittent SC and IP injections of cocaine (20 mg/kg: total of 21 injections, 430 mg/kg for each mouse) for 18 days resulted in locomotor stimulation of mice upon challenge with cocaine on the 25th or 26th day, compared with no locomotor stimulation in a saline-pretreated group. In contrast to the sensitization by intermittent cocaine administration, tolerance was found upon challenge after continuous administration of cocaine by minipumps implanted SC (25 mg/kg/day; total 450 mg/kg for each animal) on a similar schedule (Reith et al. 1987). Therefore, the mode of administration, intermittent vs. continuous, is an important factor determining the behavioral response at the endpoint.

(3) No differences were found between the above sensitized and tolerant groups in the levels of cocaine and benzoylecgonine in plasma and brain 12 minutes after IP administration of a challenge dose of cocaine, suggesting that, in these chronic experiments, the changes in the locomotor response are not accounted for by the dispositional effects. In contrast, in animals treated daily for 2 or 3 days IP with cocaine and challenged with cocaine 1 day later, there was both a greater locomotor stimulation and a higher level of cocaine in brain than in saline-pretreated animals, suggesting a dispositional effect. Among individual animals, there was a positive correlation between their locomotor stimulation by the challenge dose and their brain concentration (Reith et al. 1987). It suffices to consider the brain level of cocaine alone for dopamine-related effects of cocaine on locomotion, because the only active metabolite in this respect, norcocaine, reaches concentrations in the brain that are less than 20 percent of those of cocaine at all timepoints after IP administration of cocaine (Benuck et al. 1987).

At the same time that levels of cocaine and norcocaine peak in the brain after IP administration of cocaine, another metabolite reaches its highest concentration in the brain: N-hydroxynorcocaine. The amounts of this metabolite are at least as high as norcocaine itself

(Benuck et al. 1988). This is of considerable interest because, in liver, N-hydroxynorcocaine has been found to be the precursor of norcocaine nitroxide, a free radical that produces liver toxicity (Shuster et al. 1983).

CONCLUSION

The potent effect of cocaine in inhibiting uptake of monoamines has been known for a long time. A new approach for studying how cocaine interacts with these uptake systems has opened up with the development of receptor-binding assays of [^{3}H]cocaine binding. The binding assays are a measure of the site where cocaine acts to inhibit monoamine uptake, enabling us to study the relationship between this site and the substrate recognition site where the monoamine binds before it is translocated. The interesting possibility exists that these sites are distinct and are coupled by an allosteric linkage. If so, it is possible to envision (1) a putative cocaine antagonist that prevents the action of cocaine but does not interfere with the normal uptake of monoamines; and (2) regulation of monoamine uptake by a site that is acted upon by an endogenous ligand different from the monoamine itself. Evidence for regulation has come from studies by Hanbauer and colleagues (1985) with the dopamine transporter. In our laboratory, we have not been successful in obtaining evidence to support the allosteric model, as illustrated by the following observations. First, the absolute potencies of cocaine congeners and antidepressant drugs in inhibiting [^{3}H]cocaine binding agree closely with those in inhibiting monoamine uptake into synaptosomes, consonant with the simple model in which drugs bind to the same site as the monoamine but are not themselves translocated. Second, for most inhibitors, the competition curves in the binding and uptake assays are steep, displaying Hill slopes not very different from unity. Third, no evidence was found for changes in the dissociation rate of [^{3}H]cocaine-binding as a result of the presence of monoamines, an effect that is often associated with allosteric systems. Fourth, long-lasting or irreversibie blockade of [^{3}H]cocaine binding sites in the striatum by metaphit, an isothiocyanate derivative of PCP, causes a decrease in both [^{3}H]cocaine-binding and [^{3}H]dopamine uptake.

Like other local anesthetic drugs, cocaine inhibits sodium flux through voltage-regulated sodium channels. The blockade of flux is probably not due to mere occlusion of the channel, but involves an allosteric effect in shifting the equilibrium between the nonconducting and conducting state of the sodium channel towards the nonconducting state. This state is associated with closed gates, blocking

passage of sodium, and has a low affinity for [^{3}H]BTX-B associated with a rapid dissociation of [^{3}H]BTX-B from its binding site. The decrease in polyphosphoinositide turnover by cocaine's inhibition of sodium influx possibly interferes with processes further downstream in the sequence of events triggered by second messengers generated by inositide hydrolysis. Changes in protein kinase C-catalyzed protein phosphorylation by cocaine in the brain could be important for processes specific for the central nervous system, such as learning-induced alterations in potassium channel conductance involving second messenger systems.

It is clear that there are many more sites of action of cocaine than there are binding sites with high affinity for [^{3}H]cocaine. Furthermore, there are probably more binding sites for [^{3}H]cocaine than have been demonstrated so far. It is possible that there are discrete areas in the brain where [^{3}H]cocaine binds with characteristics different from those of the known sites. For instance, recent reports of self-administration of cocaine in the frontal cortex make it of interest to reinvestigate [^{3}H]cocaine-binding in this area. The behavioral models described above allow us to compare animals that are sensitized or tolerant to the locomotor stimulatory effect of cocaine, and it will be of great interest to study the regulation of [^{3}H]cocaine binding sites relating behavioral and biochemical measures derived from the same animal.

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Modulation of Cocaine Receptors

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INTRODUCTION

The understanding of the neuronal sites of action of cocaine are of great importance since cocaine is widely abused and produces, in man, behavioral abnormalities, as well as vascular and cardiac toxicity. The main pharmacological actions of cocaine are local anesthetic and central stimulant. In neuronal tissue, cocaine blocks the uptake of catecholamines and serotonin. This action was thought to be important to explain its behavioral effect because it altered central synaptic transmission. Cocaine has been demonstrated to bind to specific sites on brain membranes (Reith et al. 1980), where it can be displaced by compounds that fall in the pharmacological categories of nonamphetamine stimulants and antidepressants. Although the presence of saturable binding sites for cocaine was demonstrated in various types of tissues (Sershen et al. 1982), the physiological role of these sites is not well understood. At the present time, research efforts in our laboratory are focusing on several unresolved questions regarding these binding sites: (1) Does cocaine bind merely to drug acceptor sites or does it label receptors of physiological significance? (2) If cocaine binding sites are physiological receptors, are they linked to specific transducer systems? and (3) Does cocaine bind to recognition sites for an endogenous ligand?

MODULATION OF COCAINE BINDING SITES BY INORGANIC IONS

Reports in the literature indicated that the characteristics of the specific binding of a number of radioligands changed with the presence of specific ions (Enna and Snyder 1975; Baudry and Lynch 1981; Briley and Langer 1981). Studies on the effect of various ions on the specific binding of cocaine on synaptosomal membranes of

caudate nucleus are summarized in table 1. The presence of Na+ elicited a half-maximal increase in binding at about 11 mM NaCl and a maximal increase at about 25 to 60 mM NaCl. In contrast, other ions, including Li^+, K^+, and Ca^{2+} reduced cocaine binding to striatal

TABLE 1. *Modulation of specific ^{3}H-cocaine binding by inorganic substances*

Type of Ion (50 mM)	Brain Area	^{3}H-Cocaine Binding
Na^+	Caudate Nucleus (dopaminergic terminals)	↑
Na^+	Hippocampus	↓
	Frontal Cortex	↓
	Cerebellum	↓
	Hypothalamus	↓
K^+	Caudate Nucleus	↓
Ca^{2+}	Hippocampus	↓
Mg^{2+}	Cerebellum	↓

NOTE: Cocaine binding was measured in the presence of 100 nM ^{3}H-cocaine in 50 mM tris buffer, pH 7.7. Nonspecific ^{3}H-cocaine binding was measured in the presence of 50 μM cocaine.

membrane preparations (Kennedy and Hanbauer 1983). This facilitation of cocaine binding by Na^+ appeared to be limited to only one region of the brain, namely, the caudate nucleus, while in other areas, including frontal cortex, hippocampus, and cerebellum, cocaine binding was inhibited by the presence of Na^+. Kinetic analysis of saturation isotherms for cocaine binding demonstrated that in the presence of 50 mM NaCl the number of ^{3}H-cocaine binding sites was increased without altering the affinity of these sites for cocaine (Kennedy and Hanbauer 1983). Since Na^+ increased ^{3}H-cocaine binding exclusively in caudate nucleus, it was of interest to determine whether Na^+-dependent cocaine binding was linked to a specific neuronal site in this brain area. The effect of specific neuronal

lesions is summarized in table 2. Intrastriatal injection of kainic acid, a neurotoxin that selectively destroys nerve cell bodies but spares nerve endings, failed to alter Na^+-increased cocaine binding. In contrast, intraventricular infusion of 6-hydroxydopamine, a

TABLE 2. *Effect of specific neuronal lesions on Na^+-dependent cocaine binding to striatal membranes*

Type of Lesion	^{3}H-Cocaine K_D(μM)	^{3}H-Cocaine B_{Max} (pmol/mg protein)	Dopamine (pmol/mg protein)
Sham-Operated	0.34±0.02	13.4±1.6	937±34
6-Hydroxydopamine	0.37±0.06	3.7±0.3*	159±61*
Kainic Acid	0.34±0.08	14.0±1.5	nm

*p<0.001 when compared with sham-operated group.

KEY: nm=not measured.

NOTE: Rats were injected with desmethylimipramine (25 mg/kg IP) before infusing 6-hydroxydopamine (250 μg/20 μl) in 0.9 percent saline and 0.1 percent ascorbic acid. Another group of rats were infused unilaterally in the caudate nucleus with kainic acid (1 mg/2 l/rat). Cocaine binding was measured in the presence of 50 mM NaCl, 50 mM tris buffer, pH 7.7, and various concentrations of ^{3}H-cocaine (0.01 to 1.0 μM). The values represent the mean ± SE of five experiments and were obtained by Scatchard analysis of saturation isotherms.

neurotoxin that selectively destroys dopaminergic terminals, reduced the density of cocaine binding sites to a level that is obtained when measurements are carried out in the absence of Na^+. When cocaine binding in the presence of 50 mM NaCl was evaluated as a function of dopamine content, both parameters were strongly correlated (Kennedy and Hanbauer 1983). This anatomical relationship was further supported by Pimoule and coworkers (1983), who reported that the dopamine content and Na^+-dependent cocaine binding were greatly reduced in putamen of postmortem brain from Parkinson's diseased individuals when compared with putamen from non-Parkinsonians. The Na^+-dependent cocaine binding to striatal membranes was most

TABLE 3. *Displacement of ^{3}H-cocaine from specific striatal binding sites in the presence of 50 mM NaCl*

Drug	IC_{50}(M)
Nomifensine	$2x10^{-7}$
Benztropine	$5x10^{-7}$
Phencyclidine	$8x10^{-7}$
+Amphetamine	10^{-5}
SFK 38393	10^{-5}
LY 141865	$7x10^{-5}$
Dopamine	$2x10^{-5}$
Fluoxetine	10^{-5}
Tetracaine	$8x10^{-5}$
Atropine	$2x10^{-4}$

NOTE: IC_{50} values were determined by linear regression analysis of dose response curves and represent the mean of two to three different experiments. Cocaine binding was measured in presence of 100 μM ^{3}H-cocaine, 50 mM NaCl, 50 mM tris buffer pH 7.7.

potently inhibited by dopamine uptake blockers (table 3). Interestingly, dopamine itself was a relatively weak competitor for Na^+-dependent cocaine binding sites. However, the IC_{50} value for dopamine was significantly lower in the presence than in the absence of Na^+ (-NaCl: 1.9×10^{-3}M; +NaCl: 2×10^{-5}M). Specific blockers of serotonin uptake were less effective and so also were D_1 and D_2 dopamine receptor ligands, amphetamine, atropine, or tetracaine. Recently, Reith and coworkers (1985) obtained evidence that destruction of serotonergic nerve endings in caudate nucleus led to a decrease of Na^+-independent cocaine binding. If this type of lesioning absolutely spared dopaminergic terminals, this observation is of great interest, because one of the explanations given for the increased radioligand binding elicited by Na^+ was that binding to neurotransmitter uptake sites is facilitated (Baudry and Lynch 1981; Rehavi et al. 1982). If this hypothesis were to be correct, cocaine binding sites may not be functionally linked to serotonin uptake sites. But, in contrast, Reith and coworkers (1985) showed that serotonin uptake blockers were highly specific in blocking Na^+-independent cocaine binding. Thus, it appears that cocaine binding sites in caudate nucleus could be closely associated with the dopamine and

serotonin transporter systems, which may differ in their requirement for Na^+.

At the present time, the mechanism whereby Na^+ facilitates cocaine binding to dopaminergic nerve terminals is not known. Some reports in the literature on other radioligand studies attributed Na^+-induced increases in radioligand binding to stabilization of the ligand-recognition site complex (Usdin et al. 1980) or alteration in the conformation of the receptor (Pasternak et al. 1975). If cocaine binds to a transporter operative in monoamine uptake it is conceivable that Na^+ may facilitate cocaine binding by spatially exposing more transporter sites.

ENDOGENOUS MODULATORS FOR COCAINE BINDING SITES IN CAUDATE NUCLEUS

Although ample experimental evidence suggests that cocaine binding sites may play a role in the regulation of synaptic transmission by blocking neurotransmitter reuptake, there exists little published information on the regulation of cocaine binding sites. As pointed out in the previous section, in caudate nucleus, two types of cocaine recognition sites are present that differ in ion sensitivity, in anatomical localization, and in their interaction with neurotransmitter systems. Recently, our laboratory has contributed new evidence for endogenous regulatory mechanisms for the striatal Na^+-dependent cocaine binding sites. One of these mechanisms involves an interaction between dopamine and L-glutamate receptors (table 4) while the other involves a synaptosomal membrane-bound regulatory protein (Hanbauer et al. 1984) that belongs to the family of

TABLE 4. *Regulation of cocaine recognition sites in caudate nucleus*

	Na^+-Dependent	Na^+-Independent
Anatomical Localization	Dopaminergic Terminals	Serotonergic Terminals Small Neurons
Receptor-Receptor Interaction	GABA L-Glutamate	?

membrane-associated basic proteins and was found to be strikingly similar to GABAmodulin (table 5).

Receptor-Receptor Interaction

The glutamatergic neurons located in the frontal and parietal cerebral cortex project terminals into caudate nucleus, an area rich in N-methyl-D-aspartate-sensitive receptor sites (Cotman et al. 1987). Ligation of the pia mater vessels supplying blood to the frontal and parietal cortex causes destruction of these cortical layers and results in a degeneration of glutamatergic terminals in caudate nucleus, as indicated by a greatly reduced D-aspartate uptake in striatal slices (Walker and Fonnum 1983). Measurements of the Na^+-dependent cocaine binding carried out 14 days after the ligation of the pia mater vessels revealed a significant increase in the B_{max} of ^3H-cocaine binding sites in ipsilateral caudate nucleus, while the affinity for cocaine was not significantly different when compared with contralateral caudate nucleus (Sanna and Hanbauer, in preparation). The outcome of these experiments suggests that L-glutamate may exert a negative modulation on the cocaine receptors present in dopaminergic terminals. It is of interest to mention at this point that several reports in the literature (Roberts and Anderson 1979; Donzanti and Uretsky 1983; Rudolph et al. 1983) brought evidence for a dopamine receptor-glutamate receptor interaction in caudate nucleus and nucleus accumbens. In fact, L-glutamate was found to potentiate the K^+-elicited release of ^3H-dopamine in rat striatal slices. Since cocaine receptors on dopaminergic terminals appear to be functionally linked to dopamine reuptake, it is tempting to speculate that the

TABLE 5. *Extraction and purification of endogenous displacer of ^3H-cocaine binding from caudate nucleus of rats*

1. Synaptosomal fraction obtained by sucrose density gradient centrifugation.

2. Synaptosomal pellet extracted with 1 M acetic acid at 90 °C. Biogel-P10 filtration of supernatant fraction.

3. HPLC: μBondapak C_{18} preparative column eluted with linear gradient of acetonitrile containing 0.1 percent trifluoroacetic acid (15 to 40 percent acetonitrile in 50 minutes).

upregulation of cocaine receptor sites following degeneration of glutamatergic terminals may also be associated with alterations of the dopamine uptake system.

Regulation of Cocaine Binding Sites by a Synaptosomal Basic Protein

The functional and anatomical association of cocaine binding sites with the dopamine uptake system in caudate nucleus prompted us to investigate whether an endogenous modulator may exist that would interact with the binding sites to which cocaine binds with relatively high affinity. In the search for an endogenous modulator, we prepared extracts from striatal synaptosomes that were then tested for the presence of material that showed cocaine-displacing activity. Table 5 summarizes the purification steps of a cocaine binding-inhibitory protein with a molecular weight of 17,000 dalton. The final purified material was further examined by isoelectric focusing, which revealed that the cocaine binding inhibitor was a basic protein with a pI = 10.4. To obtain information on the mode of interaction of this inhibitor protein with cocaine binding sites, we measured ^{3}H-cocaine saturation isotherms in absence and presence of the inhibitor protein. The data obtained from Scatchard analysis of the ^{3}H-cocaine binding curves are shown in table 6 and clearly demonstrate that the cocaine binding inhibitor was not directly competing for ^{3}H-cocaine recognition sites but was allosterically interacting with the cocaine

TABLE 6. *Scatchard analysis of ^{3}H-cocaine binding to striatal membranes in presence or absence of cocaine binding inhibitor*

Addition to Incubation Mixture (1 ml)	^{3}H-Cocaine Binding KD (mM)	B_{Max} (pmol/mg protein)
None	0.89	11.7
Cocaine Binding Inhibitor (30 μg)	0.70	5.6

NOTE: The values represent the mean of two different measurements and were derived by Scatchard analysis of ^{3}H-cocaine binding isotherms.

binding. To characterize further the molecular nature of the cocaine binding inhibitor, we determined its amino acid composition after hydrolysis in constant-boiling HCl at 110 °C for 22 hours. The protein contained about 128 amino acid residues and had a relative abundance of *Lys* and *Arg* residues. Moreover, the relative number of the various amino acids residues/mol protein that were obtained for the cocaine binding inhibitor were very similar to those obtained for GABAmodulin (Vaccarino et al. 1985), a protein that was shown to allosterically modulate $GABA_A$ receptors (Guidotti et al. 1983). This similarity prompted us to study the effect of GABAmodulin on Na^+-sensitive ^3H-cocaine binding to striatal membranes. The data in table 7 show that GABAmodulin that was isolated from brain synaptosomes inhibited Na^+-dependent ^3H-cocaine binding with a potency comparable to that of the cocaine binding inhibitor. In fact, half-maximal inhibition of ^3H-cocaine binding was elicited by 1.7 μM of

TABLE 7. *Inhibition of NA^+-dependent ^3H-cocaine binding to striatal membranes by GABAmodulin and a cocaine binding inhibitory protein*

Addition to Incubation Mixture (1 ml)	^3H-Cocaine Bound (pmol/mg protein)
None	0.122
HPLC-Vehicle	0.140
Cocaine Binding Inhibitor (30 μg)	0.061
GABAmodulin (30 μg)	0.073

NOTE: The values represent the mean of two experiments in triplicate. GABAmodulin isolated from rat brain synaptosomes was kindly provided by Dr. A. Guidotti, FIDIA-Georgetown Institute for Neuroscience, Washington, DC.

GABAmodulin and the same degree of inhibition of ^3H-muscimol binding was achieved by 1.2 μM of the same protein. Considering the evidence on biochemical and functional similarities and the fact that

the isolation procedures were very similar, except that the cocaine binding inhibitor was extracted from synaptosomes prepared from caudate nucleus and not whole brain, it appears that GABAmodulin and the cocaine binding inhibitor are either the same protein or very similar. Moreover, the noncompetitive nature for the inhibition of the binding of both radioligands suggests that the synaptosomal basic protein may act indirectly as an allosteric modulator on recognition sites for GABA or cocaine. Previous reports in the literature demonstrated that the reduction in high-affinity binding of GABA elicited by this synaptosomal basic protein was abolished if the protein was phosphoylated *in vitro* by cyclic AMP-dependent protein kinase (Wise et al. 1983). These results suggest that phosphorylation may be a physiological mechanism to regulate the efficacy of the synaptosomal basic protein. Ongoing work in our laboratory is aimed at establishing a physiological role of this protein in the regulation of cocaine receptor sites located at dopaminergic terminals. In particular, phosphorylation of the synaptosomal basic protein or other membrane's proteins may regulate the sensitivity of cocaine recognition sites to ligands.

CONCLUSION

Studies on ion sensitivity revealed the existence of two types of cocaine binding sites: Na^+-sensitive and Na^+-insensitive sites. The Na^+-insensitive cocaine binding sites appear to be uniformly distributed in brain, but they occur in low density. The physiological role of Na^+-insensitive cocaine recognition sites and mechanisms by which such sites are regulated are unknown at the present time. The Na^+-sensitive cocaine binding sites found to be specifically located in dopaminergic terminals in caudate nucleus were found to be physiologically linked to the dopamine uptake system. The Na^+-sensitive cocaine binding sites can be allosterically downregulated by a synaptosomal basic protein, which was found to downregulate also $GABA_A$ binding sites. In addition, the density of Na^+-sensitive cocaine binding sites in caudate nucleus was increased after the destruction of glutamatergic terminals by cortical ablation. These results suggest that L-glutamate may function as a negative modulator of cocaine binding sites located in dopaminergic nerve terminals.

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Sensitization to Cocaine in the Nigrostriatal Dopamine System

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INTRODUCTION

Cocaine is a widely abused drug presumably because of its stimulant and euphoric properties. Rats given a moderate dose of cocaine show increased locomotor activity and stereotypic behavior. These behaviors are thought to be mediated by the mesolimbic and nigrostriatal dopamine (DA) pathways, respectively (Costal and Naylor 1977). Our interest in understanding the mechanisms underlying the action of cocaine in the brain was sparked by two observations reported in the literature involving these central dopaminergic systems. The first observation was that repeated cocaine administration resulted in sensitization of both locomotor activity and stereotypic behavior (Stripling and Ellenwood 1977; Post and Contel 1983). This is in contrast to repeated administration of other drugs that result in tolerance, for example, anticholinesterase agents (Overstreet and Yamamura 1979). Second was the observation that repeated cocaine administration, like that of other direct or indirect DA receptor agonists, did not consistently downregulate postsynaptic striatal DA receptors (Creese and Sibley 1981). This finding is also in contrast to other receptors such as brain muscarinic cholinergic receptors that show consistent downregulation in response to chronic agonist stimulation induced by anticholinesterase drugs (Overstreet and Yamamura 1979). Thus, repeated activation of DA pathways by cocaine may actually result in increased activity of dopaminergic systems leading to behavioral sensitization in response to subsequent cocaine administration.

A similar hypothesis has been proposed based on the results of studies demonstrating behavioral sensitization after repeated amphetamine treatment. Robinson and Becker (1986) have suggested that

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development of behavioral sensitization to amphetamine in the nigrostriatal DA pathway is not due to an increase in DA synthesis or postsynaptic DA receptors, but rather to enhanced release of DA upon reexposure to amphetamine. They also concluded that enhanced DA release after repeated amphetamine treatment did not appear to be due to DA autoreceptor subsensitivity. It is possible that a similar augmentation of DA release underlies cocaine-induced sensitization involving the nigrostriatal DA system. To address this question, we measured stereotypic behavior and neurochemical parameters of DA activity in the nigrostriatal pathway in rats treated with either single or repeated injections of cocaine. The results from these studies, as well as those in the literature, will be discussed in this chapter.

When reviewing the literature, it is essential to distinguish between changes in parameters in both cell body and terminal regions and changes in pre- and postsynaptic receptors because it is probable that these are regulated in different ways. For example, the striatum, or terminal region of the nigrostriatal tract, has been the focus of the majority of the studies. It has been suggested, however, that regions containing the cell bodies such as the substantia nigra may be more important in the development of sensitization than terminal regions. Behavioral sensitization to cocaine occurs only when repeated injections are made directly into substantia nigra, as opposed to striatum (Kalivas and Weber 1987). It is also likely that pre- and postsynaptic DA receptors would be regulated in a different fashion by repeated exposure to cocaine. Increased dopaminergic activity could be explained either by decreased activation of DA autoreceptors on either cell bodies or terminals or by increased activation of postsynaptic DA receptors. On the other hand, in order to compensate for higher levels of synaptic DA, autoreceptor activity would be expected to increase, thereby more efficiently inhibiting firing or release, and the density, affinity, or coupling of postsynaptic receptors would be expected to decrease. As mentioned previously, however, DA receptors, unlike other neurotransmitter receptors, do not always downregulate in response to chronic agonist exposure. These points will be considered below.

It is important to consider cocaine-induced changes in DA neurotransmission in the mesolimbic system in addition to those in the nigrostriatal system. Sensitization occurs to the locomotor-activating effect of cocaine, and it is known that this behavior is mediated by the mesolimbic system. However, fewer neurochemical studies have focused on this system, perhaps because of problems in working with

smaller, more diffuse brain areas or because of the more complicated behavioral changes mediated by this system. The euphoric effects of cocaine, as well as the locomotor-activating effects, are mediated by the mesolimbic system. Tolerance has been reported to occur to the euphoric effects with repeated administration (Fischman et al. 1985). Thus, the mesolimbic system represents a more complex model in which to investigate dopaminergic changes underlying sensitization to cocaine. For this reason, we have focused our own work and this chapter on the nigrostriatal system but discuss the literature pertaining to the mesolimbic system as a future direction for cocaine research.

SINGLE-DOSE ADMINISTRATION OF COCAINE

Sensitization of DA-mediated behaviors has been shown to occur after only one injection of either amphetamine (Robinson et al. 1982) or cocaine (Lin-Chu et al. 1985). Thus, one would expect that regulation of DA-containing systems would occur in response to a single dose of cocaine and, additionally, would begin in response to the first dose of cocaine in a series of repeated exposures. When investigating the effects of repeated administration of cocaine, we measured neurochemical parameters 24 hours after the last cocaine injection. Therefore, initially, we measured the effect of a single moderate dose of cocaine (10 mg/kg, intraperitoneally) injected 24 hours prior to sacrifice on several parameters of DA transmission. These parameters included tritium release elicited by either electrical stimulation or brief exposure to amphetamine from striatal slices preloaded with ^{3}H-DA, the ability of D-2 DA autoreceptors to modulate the electrically evoked release, and the number and affinity of postsynaptic striatal D-2 DA receptors. When a change was found, the duration of the change following a single injection was investigated.

Presynaptic Parameters

DA release. As already mentioned, studies of behavioral sensitization to amphetamine have supported the hypothesis that increased activity of central DA systems underlies the enhanced responsiveness to subsequent drug administration. Sensitization of both stereotypic and locomotor behavior and of amphetamine-induced release of striatal DA has been shown to develop in response to a single injection of amphetamine, and these investigators have concluded that sensitization to amphetamine in the nigrostriatal tract is predominantly due to

this increased DA release (Robinson and Becker 1986). The augmented behavioral responsiveness and neurotransmitter release are present 24 hours after acute exposure and have been shown to persist for at least 2 weeks (Robinson et al. 1982).

Likewise, both behavioral sensitization of the nigrostriatal DA system (Lin-Chu et al. 1985) and increased amphetamine-induced ^{3}H-DA release from striatal slices (Peris and Zahniser 1987) occur after a single dose of cocaine. The increase in amphetamine-stimulated release of ^{3}H-DA is present from 24 hours through 2 weeks after cocaine injection but returns to control levels after 1 month. Tritium overflow evoked by 300 pulses of electrical stimulation of slices from animals injected once with cocaine is identical in cocaine- and saline-injected animals at all of these timepoints (Peris and Zahniser 1987). It should be noted that amphetamine causes tritium overflow from tissue preloaded with ^{3}H-DA that consists primarily of ^{3}H-DA (Parker and Cubeddu 1986b), while electrical stimulation of such tissue in the absence of uptake blockers and monoamine oxidase inhibitors causes tritium overflow that consists primarily of ^{3}H-dihydroxyphenylacetic acid (^{3}H-DOPAC) (Parker and Cubeddu 1985). Thus, we will refer to ^{3}H-DA overflow in response to amphetamine stimulation but tritium overflow in response to electrical stimulation. Since amphetamine and electrical stimulation do not evoke release from the same pools (Niddam et al. 1985; Parker and Cubeddu 1986a; Parker and Cubeddu 1986b), the results reported by Peris and Zahniser (1987) suggest a redistribution of DA in the behaviorally sensitized animals into the pool(s) susceptible to release by amphetamine. Although amphetamine and cocaine may produce their *in vivo* effects on DA nerve terminals primarily via different mechanisms, i.e., amphetamine via promotion of release and cocaine via inhibition of uptake, the resulting increase in synaptic levels of DA may ultimately result in the same regulatory changes being induced in the intraneuronal pools of DA and receptor and/or transporter proteins. Thus, as Robinson and Becker (1986) have suggested for amphetamine-induced sensitization, the increase in DA-mediated behaviors induced by previous exposure to cocaine may be related to increased striatal DA release in these animals.

Cocaine has several different pharmacological actions in the central nervous system. It is an inhibitor of DA uptake, an indirect-acting DA receptor agonist, and a local anesthetic. In order to determine which of these actions is responsible for the long-lasting cocaine-induced increase in amphetamine-stimulated ^{3}H-DA release previously observed (Peris and Zahniser 1987), release was measured either 1 day

or 1 week after animals received a single injection of various substances sharing one or more of these properties of cocaine. Substances tested were mazindol, a DA uptake inhibitor and indirect-acting DA receptor agonist with no known local anesthetic properties; apomorphine, a direct-acting DA receptor agonist; and lidocaine, a local anesthetic. Additionally, in order to evaluate the contribution of the indirect receptor agonist activity of cocaine, the effects produced by a single injection of cocaine were measured after pretreatment with DA receptor antagonists.

The methods for measuring ^{3}H-DA release from rat striatal slices have been described previously (Peris and Zahniser 1987). A single injection of cocaine augments amphetamine-stimulated DA release 24 hours after the injection (Peris and Zahniser 1987). We measured the effects of mazindol (1 or 10 mg/kg), apomorphine (0.5 or 5 mg/kg), or lidocaine (20 mg/kg) on DA release evoked by 2.5-minute superfusion with 6 μM amphetamine 24 hours after a single injection. It should be kept in mind that many of these drugs have longer half-lives than cocaine; therefore, the results 1 day after injection may have been confounded. For example, the higher dose (10 mg/kg) of mazindol decreased amphetamine-stimulated release at 24 hours and increased electrically stimulated release, indicating that mazindol was still present in the striatal slices during the release experiments. Neither lidocaine nor apomorphine was effective in augmenting release after 24 hours; however, pretreatment with DA receptor antagonists abolished the effect of cocaine at 24 hours. These data suggest that the indirect agonist effect of cocaine is necessary for the augmentation of release, although one would have expected apomorphine to augment release in a manner similar to cocaine. In any case, the effect of a single injection of cocaine that remains 24 hours later should be taken into account in interpreting data from repeated cocaine administration studies in which animals were sacrificed 24 hours after the last dose of cocaine.

The effects of a single injection of cocaine and the drugs mentioned above were also examined 1 week after the drugs were injected (figure 1). As previously reported (Peris and Zahniser 1987), *in vivo* pretreatment with cocaine produced a long-lasting 25 percent increase in amphetamine-stimulated release. In comparison, pretreatment with lidocaine did not increase release significantly, whereas pretreatment with apomorphine or mazindol increased release to a similar extent as the pretreatment with cocaine. The results of the experiments examining the effects of the pretreatment with DA receptor antagonists are also shown in figure 1. When the nonselective DA

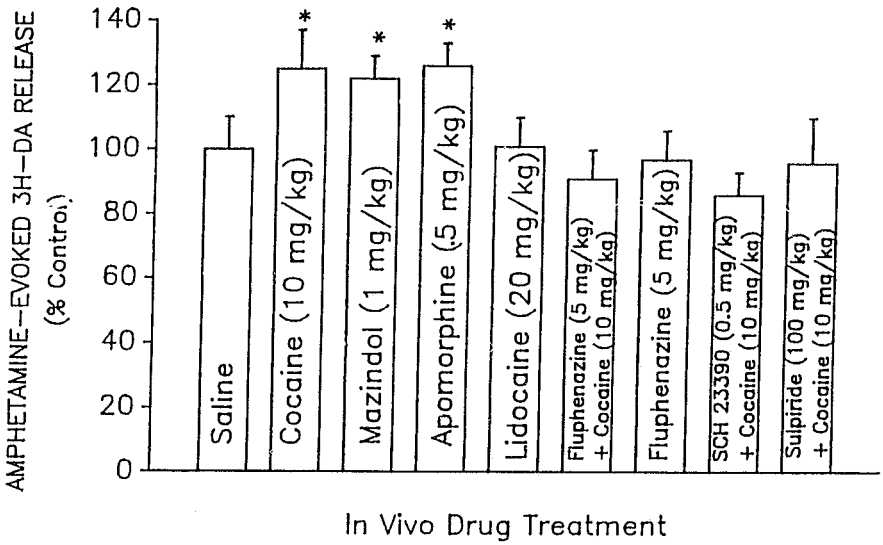

FIGURE 1. *D-amphetamine-induced ^{3}H-DA release is augmented 1 week after a single cocaine or apomorphine injection; this augmentation is blocked by DA receptor antagonists*

NOTE: Rats were injected with the drugs indicated and sacrificed 1 week later. ^{3}H-DA release was measured from striatal slices in response to exposure to 6 μM amphetamine. n=7 (saline), n=5 (mazindol, apomorphine, lidocaine), n=6 (all other treatments).

receptor antagonist fluphenazine (5 mg/kg) was injected 15 minutes prior to the cocaine administration, the long-lasting potentiation of amphetamine-induced ^{3}H-DA release produced by cocaine was abolished. Pretreatment with either the selective D-1 DA receptor antagonist SCH 23390 (0.5 mg/kg) or the selective D-2 DA receptor antagonist sulpiride (100 mg/kg) was equally effective in blocking the effects of cocaine on amphetamine-stimulated release. Similar results were found when ^{3}H-DA release was evoked by superfusion with either 2 or 20 μM amphetamine (data not shown). None of the antagonists had an effect on release when injected without cocaine. Furthermore, pretreatment with any of the drugs did not affect tritium overflow evoked by electrical field stimulation (5 Hz, 1 minute, 300 pulses) from these slices in the same experiments (data not shown).

These results suggest that the indirect agonist effects of cocaine are involved in producing the enhanced amphetamine-evoked release observed after a single dose of cocaine. Apomorphine, a direct-acting DA receptor agonist, mimics the effects of cocaine, and DA receptor antagonists block the effects of cocaine. As expected, mazindol produces the same effects as cocaine because mazindol is also an indirect DA receptor agonist. However, one difference between cocaine, apomorphine, and mazindol is their duration of action in the brain. Cocaine and apomorphine are essentially completely cleared from brain within 60 minutes (Nayak et al. 1975; Schwartz et al. 1978), while mazindol has a much longer half-life in plasma of 30 hours (Shindler et al. 1985). It is likely that the effects of these drugs at 24 hours after injection differ because of the different half-lives. These drugs also differ in their effects on different amine neurotransmitters. Cocaine inhibits uptake with the following order of potencies: serotonin > DA > norepinephrine, while the order of potencies for mazindol is norepinephrine > DA = serotonin (Ritz et al. 1987). Apomorphine, on the other hand, is a selective agonist for DA receptors. These differences will be explored in future experiments. Additionally, it appears that activation of both D-1 and D-2 DA receptors is necessary for cocaine-induced sensitization to occur. Along these same lines, apomorphine activation of locomotor behavior can be blocked by either D-1 or D-2 receptor antagonists (Schwartz et al. 1978; Herrera-Marschitz and Ungerstedt 1985; Amalric et al. 1986; Vaccheri et al. 1986). As has been suggested recently for a number of DA receptor-mediated behaviors (Gershanik et al. 1983; Braun and Chase 1986; Arnt et al. 1987; Carlson et al. 1987; Wachtel et al. 1987; Walters et al. 1987), D-1 receptors play an additive, a permissive, or even an obligatory role in D-2 receptor-mediated events.

If the indirect-acting agonist effects of cocaine underlie the sensitization to amphetamine-evoked release, then these effects may also be the basis for the behavioral sensitization seen in response to a single injection of cocaine. It would be of interest to measure whether stereotypic behavioral sensitization develops to cocaine if rats are pretreated with selective D-1 and D-2 DA receptor antagonists prior to the initial dose of cocaine. Previous attempts to block behavioral sensitization to repeated cocaine injections with nonselective DA receptor antagonists have resulted in either complete (Borison et al. 1979; Beninger and Herz 1986) or partial blockade (Gale 1984).

DA Uptake, Synthesis, and Metabolism. Despite the fact that many of the effects of cocaine can be explained by its ability to block

reuptake of DA into the nerve terminal, we have found no changes in either the affinity or the maximal velocity of neuronal ^{3}H-DA uptake into striatal slices 24 hours after pretreatment with a single 10 mg/kg injection of cocaine (Zahniser et al. 1986). ^{3}H-nomifensine can be used to label specific recognition binding sites for the DA uptake pump (Dubocovich and Zahniser 1985). Saturation analysis of specific ^{3}H-nomifensine binding to striatal membranes of rats treated 24 hours earlier with cocaine also revealed no differences when compared with controls (Zahniser et al. 1986). Thus, no persistent changes appear to be induced in the neuronal DA uptake pump by a single injection of cocaine. When neuronal ^{3}H-DA uptake is measured *in vitro* 20 minutes after cocaine is administered *in vivo*, there is, as expected, a dose-related decrease in striatal DA uptake (Missale et al. 1985). However, by 60 minutes after cocaine administration, uptake returns to control levels (Missale et al. 1985). These results are consistent with the 20-minute half-life of cocaine in the brain (Nayak et al. 1975).

Tissue levels of DA are not changed in the striatum between 3 and 24 hours after a single injection of cocaine (Taylor and Ho 1977; Zahniser et al. 1986; Hanson et al. 1987), indicating that cocaine is probably not having a neurotoxic effect. Cocaine increases striatal tyrosine hydroxylase activity up to 40 minutes after cocaine injection (Pradhan 1983) but has no effect 3 hours after injection (Hanson et al. 1987). The level of DOPAC, one of the major metabolites of DA, decreases in the striatum 60 minutes after an acute injection of cocaine (Kalivas et al. 1988). The levels of the other major metabolite, homovanillic acid, or HVA, are not altered in striatum 30 minutes after a single cocaine injection (Taylor and Ho 1977) but have been reported to increase 3 hours after injection (Hanson et al. 1987). Thus, the short-term effects of a single injection of cocaine on DA synthesis and metabolism are complex, but there appears to be an overall decrease in both intraneuronal and extraneuronal DA metabolism (Bagchi 1986). In general, an increase in synthesis and decrease in metabolism caused by cocaine should result in an overall transient increase in synaptic concentrations of DA after a single injection of cocaine. However, this effect does not persist long enough to make it a good candidate to explain sensitization to cocaine.

D-2 DA Autoreceptors. D-2 DA receptors localized on DA neurons are termed autoreceptors. The following three types of DA auto-receptors have been proposed to be associated with dopaminergic nigrostriatal neurons: impulse-regulating autoreceptors associated

with cell bodies and synthesis- and release-modulating autoreceptors localized on the nerve terminals (Jackisch et al. 1980; Bannon and Roth 1983). The activity of the first two types of nigrostriatal DA autoreceptors has not been reported following a single injection of cocaine. The activity of striatal D-2 release-modulating autoreceptors can be measured using an *in vitro* superfusion system, by determining the ability of the D-2 receptor agonist pergolide to inhibit the tritium overflow evoked by electrical field stimulation from striatal slices preloaded with ^{3}H-DA (Dwoskin et al. 1988). These receptors must be studied with a functional assay because they have not been detected using radioligand binding assays either with striatal membranes and conventional filtration assays (Zahniser et al. 1986) or with striatal sections and quantitative autoradiography (Filloux et al. 1988). The activity of these striatal D-2 release-modulating autoreceptors is not different when measured 24 hours after one injection of cocaine (Dwoskin et al. 1988). Therefore, it is unlikely that changes in these D-2 release-modulating autoreceptors could explain the increased behavioral sensitivity to cocaine seen after one injection (Lin-Chu et al. 1985). Indeed, of all the presynaptic parameters investigated, only increases in amphetamine-induced release of DA and possible increases in synthesis of DA coupled with decreases in metabolism could underlie behavioral sensitization.

Postsynaptic Parameters

D-1 and D-2 DA Receptors. Only a few studies have measured the effect of one cocaine injection on DA receptor binding properties. An increased density of D-2 DA receptors in striatum with no effect on receptor affinity has been observed 60 minutes after a single injection of cocaine (Memo et al. 1981). However, when striatal D-2 receptors were measured 24 hours after one injection of cocaine (10 mg/kg), no differences were seen in either receptor density or affinity (Dwoskin et al. 1988). Thus, once again it appears as if there is no long-lasting change in postsynaptic striatal D-2 DA receptors that could explain the persistent behavioral changes seen after a single exposure to cocaine. To our knowledge, the effects of a single injection of cocaine on striatal D-1 DA receptors have not been examined.

Second Messengers. DA acting at postsynaptic DA receptors in the striatum exhibits dual regulation of adenylate cyclase activity. Activation of D-1 DA receptors stimulates cAMP production, while activation of D-2 receptors inhibits cAMP formation (Stoof and Kebabian 1981; Cooper et al. 1986). DA exhibits a decreased potency

in stimulating striatal cAMP formation 60 minutes after one cocaine injection (Memo et al. 1981). It is interesting to note that acute administration of amphetamine promotes a rapid, transient desensitization of striatal DA-stimulated adenylate cyclase activity (Barnett and Kuczenski 1986). These findings may be interpreted as either a decrease in D-1 receptor-mediated stimulation of adenylate cyclase activity or an increase in D-2 receptor-mediated inhibition. Long-lasting effects of acute exposure to cocaine on striatal adenylate activity have not yet been measured.

REPEATED ADMINISTRATION OF COCAINE

Behavioral sensitization to the locomotor activating and stereotypic effects of repeated cocaine administration is well-documented in rats (Stripling and Ellenwood 1977). The degree of sensitization is also augmented with repeated exposure to cocaine, and this expression of sensitization may therefore involve mechanisms additional to those involved following a single injection of cocaine. More studies have focused on measuring parameters that may explain behavioral sensitization following repeated, as opposed to single dose, administration of cocaine. Since activation of the nigrostriatal dopaminergic pathway is implicated in these stereotypic behaviors, it has again been reasoned, as in the single-dose administration studies, that there may be a concomitant change in either pre- or postsynaptic parameters of DA neurotransmission in this pathway leading to greater activation and explaining sensitization.

Presynaptic Parameters

DA Release. Based on our findings of a long-lasting increase in amphetamine-induced release of ^{3}H-DA from striatal slices of rats treated with only one dose of cocaine (10 mg/kg), we expected to find an even greater augmentation following repeated administration of cocaine. In contrast to the acute injection results, however, following either 8 or 14 once-daily injections of 10 mg/kg cocaine, amphetamine-induced ^{3}H-DA release was not different from saline-treated animals (unpublished observations). This finding was puzzling because our previous results showed that release was increased for 2 weeks following a single injection of cocaine. Thus, in these experiments, we expected that release would be increased in response to the last injection of cocaine for the same duration. However, recent data indicate that if animals are sacrificed 72 hours after the last dose of cocaine, then amphetamine-stimulated ^{3}H-DA release is increased twofold over water-injected controls (Johnson and Snell

1987). Thus, it appears that a compensatory change that obscures the increase in amphetamine-induced release occurs after repeated cocaine injections, but that this compensatory mechanism disappears within a few days of terminating the cocaine administration. One possible compensatory change involves the D-2 release-modulating autoreceptors in the striatum. It is controversial whether D-2 autoreceptors are capable of modulating amphetamine-induced release (Kamal et al. 1981; Kelly and Nahorski 1987). We have preliminary evidence that the D-2 receptor antagonist S-sulpiride increases ^{3}H-DA release evoked by low concentrations of amphetamine but not that evoked by high concentrations. If the activity and/or density of these autoreceptors were increased in response to the repeated cocaine injections (as discussed below), then these receptors could inhibit DA release to a greater extent in striatum of cocaine-treated animals. This hypothesis could be tested by determining whether blockade of D-2 autoreceptors during measurement of amphetamine-induced ^{3}H-DA release from cocaine-sensitized animals reinstates the augmented release seen after one injection of cocaine. When augmented amphetamine-evoked DA release was seen after repeated amphetamine administration, measurements of release occurred 10 days after the last amphetamine exposure, thereby allowing some degree of withdrawal to take place (Robinson and Becker 1986). It is possible that if release were measured 24 hours after the last amphetamine injection, augmentation of release would not have occurred.

Dendrites release DA, which can interact with impulse-regulating autoreceptors in the cell body region. When rats were injected with cocaine (15 or 45 mg/kg) for 3 days and then withdrawn for 2 to 3 weeks, a decrease in both amphetamine- and K^{+}-stimulated release of endogenous DA was observed in ventromedial mesencephalon, the region containing the cell bodies of the nigrostriatal, mesolimbic, and mesocortical DA-containing pathways (Kalivas and Duffy 1988). This decreased somatodendritic release could result in less autoreceptor inhibition at the cell body, thereby increasing the firing of the nigrostriatal tract and DA release from the terminals. In fact, Kalivas and Duffy observed an increased release in striatum only after the animals had been withdrawn from the higher dose of cocaine. This alteration is consistent with sensitization and stresses the importance of cocaine-induced changes that may occur at the cell body. It is also interesting to note that changes that occur that are consistent with the development of behavioral sensitization are long-lasting and may become apparent only after a certain drug-free period.

DA Uptake, Synthesis, and Metabolism. No changes in the neuronal uptake of ^{3}H-DA in the striatum were observed 20 minutes after withdrawal from repeated cocaine administration (Missale et al. 1985). However, acute cocaine decreases the V_{Max} of ^{3}H-DA uptake in striatum; therefore, repeated administration can be said to result in the development of tolerance to the ability of cocaine to block uptake (Missale et al. 1985). Consistent with the findings of this group, we also observed no differences in the properties of either high-affinity ^{3}H-DA uptake or ^{3}H-nomifensine binding in striatal tissue from behaviorally sensitized animals that had been treated for 8 or 14 days with cocaine and then withdrawn for 24 hours (Zahniser et al. 1986). Taken together, these results suggest that in relation to the uptake blockade produced by cocaine in the striatum, there may be less of an increase in synaptic DA levels produced in response to repeated cocaine exposure. This finding is consistent with the development of tolerance rather than sensitization.

Striatal DA levels are the same as those in control rats after a 24-hour withdrawal from repeated cocaine treatment (Zahniser et al. 1986; Yeh 1987). This indicates that repeated administration of moderate doses of cocaine does not produce DA neurotoxicity. Following 9 days of repeated cocaine injections, there is an increase in DA content in both terminal and cell body regions when DA is measured 20 minutes, but not 60 minutes, after the last cocaine injection (Roy et al. 1978). This increase is no longer present on the 18th or 30th day of repeated cocaine injections. Chronic cocaine decreases tyrosine hydroxylase immunoreactivity in both nigrostriatal cell body and terminal regions (Trulson et al. 1986). However, there has also been a report of increased striatal tyrosine hydroxylase activity after repeated cocaine injections (Taylor and Ho 1977). In the striatum, a persistent decrease in DA metabolism following repeated injections of cocaine (Karoum et al. 1987), a slight increase (Taylor and Ho 1977), or no change (Yeh 1987) have been reported. In summary, no consensus exists as to long-lasting changes in DA content, synthesis, or metabolism produced by repeated administration of cocaine.

D-2 DA Autoreceptors. As discussed previously, D-2 DA auto-receptors on terminals of the nigrostriatal tract modulate the stimulation-evoked release of DA. These autoreceptors appear to comprise an insignificant number of the D-2 receptors measured with *in vitro* radioligand binding assays. When these receptors were measured functionally, they showed a small but consistent increased sensitivity 24 hours after the 8- or 14-day cocaine treatment

(Dwoskin et al. 1988). These data are in contrast to the classical downregulation or decreased sensitivity observed for many post-synaptic receptors after prolonged agonist stimulation (Creese and Sibley 1981). An increased sensitivity of these receptors would inhibit stimulation-evoked release of DA to a greater extent and would oppose the increased concentrations of synaptic DA produced in response to cocaine. This change would also oppose increased DA release in response to decreased impulse-regulating autoreceptor activation and the component of release that is represented by increased amphetamine-induced release. Therefore, this appears to be a compensatory response consistent with the development of tolerance.

In contrast with D-2 DA receptors in the terminal fields, those in the cell body region, i.e., the substantia nigra compacta, are all impulse-regulating autoreceptors (Filloux et al. 1988). Therefore, radioligand binding assays can be used to quantitate D-2 autoreceptors in this area. We have recently used quantitative autoradiographic analysis to examine specific binding of ^{3}H-spiperone to D-2 receptors in the substantia nigra compacta and the substantia nigra reticulata of rats treated with cocaine for 8 days and withdrawn for 24 hours. The binding to a single high concentration of ^{3}H-spiperone was significantly increased in the caudal half of the substantia nigra compacta but was not changed in the substantia nigra reticulata (data not shown). If this increase in D-2 autoreceptors in the substantia nigra is functionally significant, then this cocaine-induced change, like that of the release-modulating autoreceptors in the striatum, would also be consistent with dampened dopaminergic activity and the development of tolerance to the stimulating effects of cocaine. It should be noted that the decreased somatodendritic release reported by Kalivas and Duffy (1988) could be the result of the increased D-2 autoreceptors in this area. When the data are considered in this way, they are more consistent with tolerance rather than sensitization.

Postsynaptic Parameters

D-1 and D-2 DA Receptors. Most of the studies concerned with DA receptor regulation after repeated cocaine administration have measured parameters of D-2 receptor binding. Until recently, it was generally accepted that D-2 receptors were functionally more important than D-1 receptors (Seeman 1980). Despite several studies, however, no consensus has been reached as to the modulation of D-2

receptors by repeated cocaine administration. Initial studies reported dose-related increases in striatal D-2 DA receptor number (Borison et al. 1979; Taylor et al. 1979). More recently, Goeders and Kuhar (1987), using quantitative autoradiography, found that repeated cocaine administration for 15 days resulted in a decrease in the density of striatal D-2 receptors. Using either membrane binding assays (Dwoskin et al. 1988) or quantitative autoradiography (unpublished observations), we found no differences in the affinity or density of these receptors in the striatum of rats treated for 8 or 14 days with once-daily injections of cocaine. Goeders and Kuhar (1987) sacrificed the rats 20 minutes after the last dose as compared to 24 hours or more in all the other studies. At 20 minutes, the tissue concentration of cocaine would still be relatively high, whereas by 24 hours it would be negligible. Thus, the difference in the time when the tissue was taken may explain some of the differences in the results of these studies.

The fact that we found no significant change in the density of striatal D-2 receptors may seem at odds with our functional data showing increased inhibition by D-2 autoreceptors in striatum after repeated cocaine treatment. The discrepant results are not explained by the fact that the binding studies were carried out using an antagonist, while the functional studies were carried out using an agonist. Competition curves for the agonist pergolide revealed similar binding affinities in the control and cocaine-treated rat striatal membranes (Dwoskin et al. 1988). Instead, these data support the idea that the number of autoreceptors may be a relatively small proportion of the total D-2 receptors in striatum and that for this reason the autoreceptors may not be detected by radioligand binding assays even with the added sensitivity of quantitative autoradiography.

In adjacent tissue sections in the quantitative autoradiographic studies, we used ^{3}H-SCH 23390 to label D-1 DA receptors. In the striatum, the D-1 receptors are thought to be localized postsynaptically, whereas in the substantia nigra reticulata the D-1 receptors appear to be localized on the striatonigral terminals (Altar and Hauser 1987). No changes in D-1 receptors were found in either area when the values from the control and cocaine-treated rats were compared (data not shown).

Second Messengers. It has recently been reported that the potency of DA to stimulate adenylate cyclase activity in the striatum decreases after repeated amphetamine exposure; this desensitization occurs concomitantly with behavioral sensitization (Barnett et al.

1987). Repeated treatment with the DA uptake blocker nomifensine also decreases DA-stimulated cAMP formation (Algeri et al. 1980). Chronic treatment with DA receptor agonists L-DOPA, piribedil, or bromocriptine also abolishes DA-stimulated cAMP formation in striatal slices (Mishra et al. 1978). These effects on adenylate cyclase are presumably mediated via postsynaptic D-1 DA receptors and are consistent with the development of tolerance. As yet, no one has measured the effect of repeated cocaine administration on the regulation of adenylate cyclase by either D-1 or D-2 receptors in the striatum.

FUTURE DIRECTIONS

As mentioned in the introduction, sensitization also occurs to the locomotor-activating effect of cocaine, a behavior that is mediated by the mesolimbic DA pathway. It is therefore possible that similar changes in DA function occur both in this pathway and in the nigrostriatal pathway, which mediates behavioral sensitization to the stereotypic effects of cocaine. However, changes in the mesolimbic system may be more complex because the mesolimbic, as well as the mesocortical, dopaminergic projections are implicated in the reinforcing or euphoric effects of cocaine (Wise 1981; Wise 1984) and tolerance, rather than sensitization, occurs to the euphoric effect of cocaine (Fischman et al. 1985). We will now discuss the observations made concerning cocaine-induced changes in the mesolimbic system as they pertain to the future directions of research in the cocaine field.

It is clear that at least some of the effects of cocaine differ depending on whether measurements are made in the mesolimbic or nigrostriatal pathways. Application of cocaine inhibits spontaneous firing of DA neurons in the ventral tegmental area both more potently and more effectively than it does in substantia nigra compacta (Pitts and Marwah 1987), supporting the hypothesis that cell bodies in mesolimbic and nigrostriatal DA systems are affected differently by cocaine exposure. *In vitro* cocaine inhibits DA uptake to a greater extent in striatum than in other DA terminal areas such as prefrontal cortex (Hadfield and Nugent 1983) and nucleus accumbens (Missale et al. 1985). Similar effects are observed transiently in the striatum and prefrontal cortex after *in vivo* administration of cocaine; paradoxically, *in vivo* pretreatment with cocaine transiently increases uptake into nucleus accumbens (Missale et al. 1985). Chronic cocaine administration increases the maximal velocity of ^{3}H-DA uptake into nucleus accumbens, whereas no change occurs in striatum (Missale et al. 1985). These same investigators also found

that ^{3}H-cocaine binding in nucleus accumbens is sodium ion-independent unlike that found in striatum, which is sodium ion-dependent. Thus, cocaine binding sites and/or coupling of these sites to the DA transporter may differ between the mesolimbic and nigrostriatal pathways.

Differences are also evident in the receptor regulation induced by cocaine. Unlike the nigrostriatal system, in which both the cell body and terminal D-2 autoreceptors change in a manner consistent with increased inhibitory activity and, therefore, tolerance following repeated cocaine administration, D-2 autoreceptors in the mesolimbic system change in the opposite direction. Consistent with sensitization, D-2 autoreceptors in the ventral tegmental area are subsensitive after chronic cocaine treatment (Greene and White 1986; Henry et al. 1987). Furthermore, postsynaptic D-2 receptors in nucleus accumbens are more sensitive to agonists after repeated cocaine exposure (Henry et al. 1987). This may be explained by the fact that after repeated cocaine injections, an increased density of D-2 DA receptors in nucleus accumbens has been found in two studies using quantitative autoradiographic analysis (Goeders and Kuhar 1987; unpublished observations). Thus, both pre- and postsynaptic D-2 receptors associated with the mesolimbic tract appear to be regulated differently than those in the nigrostriatal tract. Another difference between these two systems that has recently been reported suggests that cocaine might not be expected to have the same postsynaptic effects in the mesolimbic and nigrostriatal pathways. D-2 DA receptor-mediated inhibition of adenylate cyclase activity occurs in striatum and not in nucleus accumbens (Stoof and Verheijden 1986).

On the other hand, some of the effects of exposure to cocaine are similar in the nigrostriatal and mesolimbic pathways. For example, after a single injection of cocaine, DA levels remain stable in both striatum and nucleus accumbens (Henry et al. 1987). Chronic cocaine also decreases tyrosine hydroxylase immunoreactivity in terminal regions of the mesolimbic system (Babb et al. 1986; Trulson et al. 1986). Similar to the nigrostriatal pathway, cell body regions appear more important in the action of cocaine because behavioral sensitization occurs only if cocaine or amphetamine is injected into the ventral tegmental area, as opposed to the nucleus accumbens (Kalivas and Weber 1987). One other similarity is that following repeated administration of cocaine, D-1 receptors are not regulated in either the nigrostriatal or mesolimbic systems (unpublished observations).

All of these observations considered together suggest that activity in both the cell body and terminal regions of the mesolimbic and nigrostriatal pathways are modulated somewhat differently by repeated cocaine administration. Perhaps most striking are the differences in the regulation of D-2 receptors in the two pathways. In general, the receptor changes in the nigrostriatal pathway are consistent with tolerance, whereas those in the mesolimbic pathway are consistent with sensitization. It is intriguing that the majority of the changes observed in the mesolimbic pathway could be the basis for behavioral sensitization, since both sensitization and tolerance have been reported to occur for behaviors mediated by this pathway.

SUMMARY

Behavioral sensitization involving the nigrostriatal dopaminergic tract is manifested after treatment with only a single dose of cocaine and is augmented following repeated treatment. One neurochemical change observed that is consistent with behavioral sensitization is the increase in amphetamine-induced ^{3}H-DA release from striatal slices seen after one injection of cocaine. One day after repeated administration of cocaine, however, the increase is no longer evident. It is possible that transient compensatory changes, such as increased D-2 autoreceptor inhibition, may obscure this effect when it is measured at relatively short times after the repeated administration has been terminated. One day after cessation of repeated cocaine administration, D-2 autoreceptors in both striatum and substantia nigra compacta were upregulated consistent with a compensatory mechanism and the development of behavioral tolerance rather than sensitization. In contrast, DA content, neuronal DA uptake, and postsynaptic D-2 DA receptors in striatum were not regulated by this treatment. Likewise, D-1 DA receptors in striatum and substantia nigra were unaffected. In the mesolimbic system, both the pre- and postsynaptic receptor changes are consistent with sensitization. Amphetamine-stimulated release from nucleus accumbens has not yet been measured in cocaine-sensitized animals. It is possible that changes similar to those seen in striatum may occur in this area. It is interesting that, in general, presynaptic parameters associated with the DA neuron, with the notable exception of the uptake pump, appear to be more sensitive to regulation by cocaine administration than do postsynaptic parameters. The long-lasting effects of a single moderate dose of cocaine are also surprising. It will be important to determine the molecular mechanisms underlying this regulation and whether or not similar changes are induced in mesolimbic dopaminergic systems by single and repeated administration of cocaine.

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The Interaction of Cocaine With Central Serotonergic Neuronal Systems: Cellular Electrophysiologic Approaches

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The numerous behavioral and physiological effects elicited by cocaine in humans range from affective changes (euphoria and mood elevation) at low doses, to psychiatric disorders (anxiety, depression, and paranoid psychosis) at high or chronic doses (Fischman 1987). Many of these effects of cocaine have been related to the ability of cocaine to stimulate catecholamine neurotransmission in the central nervous system (CNS) (Seiden and Kleven, this volume). However, cocaine has also been demonstrated to significantly alter central serotonin (5-hydroxytryptamine [5-HT]) function (Blackburn et al. 1967; Friedman et al. 1975; Ross and Renyi 1967; Ross and Renyi 1969; Taylor and Ho 1978). Since brain 5-HT-containing neuronal systems have been demonstrated to be involved in psychiatric disorders (Ceulemans et al. 1985; Iversen 1984; King et al. 1985; Van Praag 1982), including those evidenced by abusers of this stimulant, a possible role for the interaction of cocaine with serotonergic neuronal systems warrants further investigation.

The classic pharmacological profile of cocaine includes both its actions as a local anesthetic (Ritchie and Greene 1985) and as an inhibitor of monoamine neurotransmitter reuptake. While cocaine has been recognized to potently inhibit the uptake of the catecholamines dopamine (DA) (Bagchi and Reilly 1983; Heikkila et al. 1975; Komisky et al. 1977; Nielsen et al. 1983; Taylor and Ho 1978) and norepinephrine (NE) (Hadfield et al. 1980; Komisky et al. 1977; Taube et al. 1975; Taylor and Ho 1978), a similar inhibition of reuptake also occurs for the indoleamine 5-HT (Blackburn et al. 1967; Koe 1976; Ross and Renyi 1967; Ross and Renyi 1969; Taylor and Ho 1978). Although equipotent as a reuptake inhibitor of these monoamine neurotransmitters, cocaine is actually more efficacious as an inhibitor of 5-HT uptake as compared to DA or NE uptake processes. These

data indicate that interactions with central serotonergic function may underlie some of the observed psychological and physiological effects of cocaine.

Studies of the content and turnover of 5-HT after acute or chronic cocaine administration also suggest the importance of cocaine-5-HT interactions. Several groups have reported brain levels of 5-HT to be unchanged following administration of cocaine (Friedman et al. 1975; Schubert et al. 1970). However, Pradhan and colleagues (Pradhan et al. 1978a; Pradhan et al. 1978b; Pradhan et al. 1981; Roy et al. 1978) have also identified significant decreases in the levels of 5-HT in several brain regions following either acute or chronic cocaine administration. In addition, several laboratories have identified decreases in 5-HT synthesis produced by this psychostimulant (Friedman et al. 1975; Galloway and Novak 1986; Post et al. 1976; Schubert et al. 1970). Furthermore, cocaine has been shown to decrease both the uptake of the 5-HT precursor tryptophan and increase the activity of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis (Knapp and Mandell 1972; Knapp and Mandell 1976). Likewise, studies of ^{3}H-cocaine binding have revealed both a differential distribution of binding sites in the brain (Kennedy and Hanbauer 1983; Reith et al. 1983; Shah et al. 1982) as well as a presynaptic labeling of DA or 5-HT reuptake sites both in the striatum (Kennedy and Hanbauer 1983; Reith et al. 1983; Reith et al. 1985; Schoemaker et al. 1985) and the cortex (Reith et al. 1983).

Behavioral studies of cocaine's actions have also implicated a role for 5-HT neuronal systems in the mediation of specific drug-induced behaviors. Inhibition of 5-HT synthesis and selective 5-HT receptor blockade have been reported to potentiate cocaine-induced locomotor activity (Scheel-Kruger et al. 1977). Conversely, administration of the precursor 5-hydroxytryptophan has been demonstrated to attenuate the locomotor activity produced by cocaine administration (Pradhan et al. 1978a). It is relevant to note that although a role for DA in mediating the reinforcing and discriminative stimulus properties of cocaine is supported by much of the behavioral research (Koob and Hubner, this volume; Woolverton and Kleven, this volume), not all of the behavioral effects of cocaine are consistently inhibited by DA antagonists (Colpaert et al. 1976; Cunningham and Appel 1982; Friedman et al. 1975; Spyraki et al. 1982). Thus, the behavioral pharmacology of cocaine suggests that interactions with other neurotransmitters are probably important in mediating the effects of cocaine.

In assessing the neuropharmacological ramifications of the interaction of cocaine with central 5-HT neuronal systems, we have begun to study the effects of cocaine administration on the spontaneous activity of serotonin neurons recorded extracellularly in the dorsal raphe nucleus (DRN) of the anesthetized rat. Such a single-unit electrophysiologic approach can help to identify the precise cellular and molecular physiological events by which cocaine may act on specific, identified neuronal populations in the brain. These serotonin-containing neurons provide a useful neurobiological model system for studies of the cellular actions of cocaine, as these DRN neurons are well characterized, both with respect to their pharmacological and physiological properties. Not only do these midbrain 5-HT cells have an identified transmitter substance (Dahlstrom and Fuxe 1964), but they are readily identified by standard electrophysiologic recording techniques and discharge spontaneously with a uniform, steady firing rate (Aghajanian 1976; Haigler and Aghajanian 1974). The synaptic projections of these 5-HT neurons have also been well characterized and include extensive innervation of all forebrain regions (Azmitia and Segal 1978; Conrad et al. 1976; Moore et al. 1978; Parent et al. 1981; Steinbusch 1981; Ungerstedt 1971). A wealth of information has accumulated with respect to the direct inhibitory effects of microiontophoretically applied 5-HT (Aghajanian 1972; Aghajanian et al. 1987; Trulson and Jacobs 1976; VanderMaelen 1985), histamine (Lakoski and Aghajanian 1983; Lakoski et al. 1984), and GABA (Gallager and Aghajanian 1976b; Wang and Aghajanian 1977) as well as excitatory effects of the microiontophoretic application of NE (Baraban and Aghajanian 1980; Gallager and Aghajanian 1976a; Menkes et al. 1981; Rogawski and Aghajanian 1982) on these neurons. The inhibitory and excitatory inputs for this presynaptic 5-HT autoreceptor located in the DRN are diagrammed in figure 1.

Systemic administration of cocaine has recently been demonstrated to inhibit the spontaneous activity of 5-HT neurons recorded in the DRN (Cunningham and Lakoski 1986; Cunningham and Lakoski 1988; Pitts and Marwah 1986b; Pitts and Marwah 1987a). A complete, dose-dependent, and reversible inhibition of the spontaneous firing of these neurons is observed following intravenous administration of cocaine (cumulative dose to 50 percent inhibition = 0.66 ± 0.11 mg/kg) (figure 2). While the central effects of cocaine may be mediated, in part, via local anesthetic properties of the drug, this is not apparent with respect to the modulation of 5-HT neuronal activity. As recorded in the DRN, intravenous administration of the local anesthetics procaine or lidocaine (Cunningham and Lakoski 1988; Pitts and Marwah 1986b) (figure 2) failed to alter spontaneous cell firing

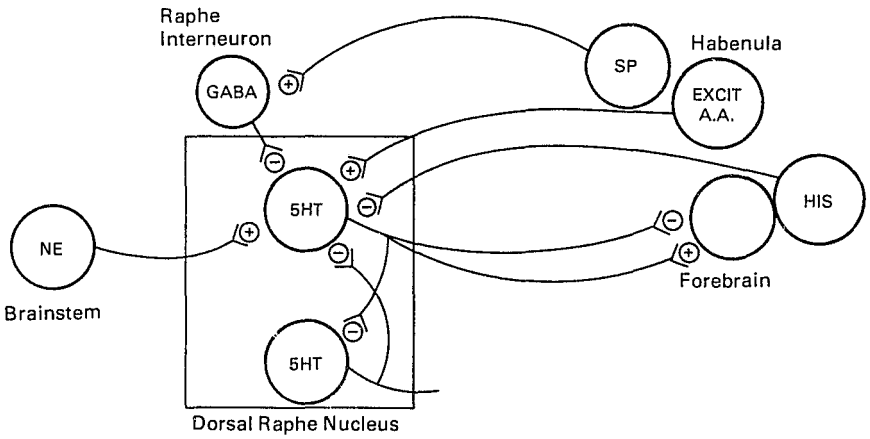

FIGURE 1. *A summary of identified physiological inputs to serotonin (5-HT)-containing dorsal raphe neurons*

KEY: NE, norepinephrine; 5-HT, serotonin; GABA, γ-aminobutyric acid; SP, substance P; EXCIT A.A., excitatory amino acids; HIS, histamine. The plus symbol (+) indicates a given substance enhances spontaneous cell firing, while a minus symbol (-) indicates a corresponding inhibition.

in a manner similar to that observed with cocaine; indeed, several neurons found to be unresponsive to procaine administration were inhibited by subsequent systemic cocaine application (Cunningham and Lakoski 1988).

Since cocaine is a potent inhibitor of 5-HT reuptake processes in brain tissues, pharmacological characterization of the effects of cocaine on DRN cell firing has involved comparison of the effects of cocaine with uptake inhibitors that are selective for DA, NE, or 5-HT. Fluoxetine, a selective 5-HT uptake inhibitor, but not the selective uptake inhibitors for NE (desipramine) or DA (GBR 12909), inhibited 5-HT cell firing in a dose-response manner (Cunningham et al. 1987b); in addition, pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine reduced the sensitivity of 5-HT DRN neurons to the systemic application of this psychostimulant). These data are consistent with the idea that depressant effects of cocaine on serotonergic cellular physiology may result from a direct inhibition of 5-HT neuronal activity as a consequence of the selective inhibition of monoamine reuptake (Lakoski and Cunningham 1988). In support

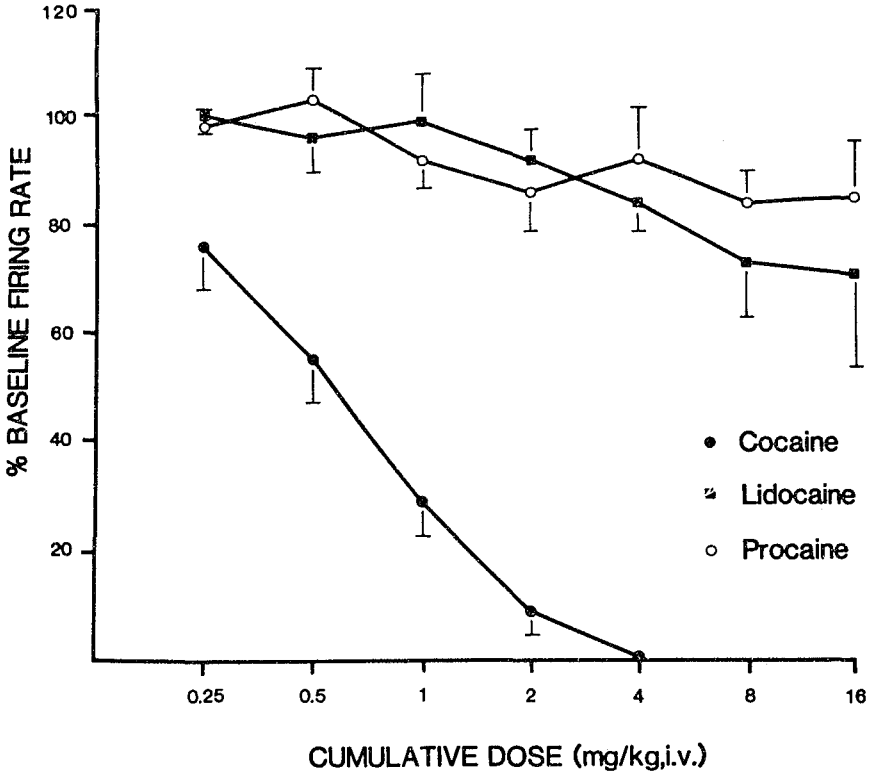

FIGURE 2. *Comparison of the effects of intravenous cocaine and local anesthetics administration on spontaneous serotonergic cell firing recorded in the dorsal raphe nucleus*

NOTE: Data are presented for the cumulative dose-response curve to cocaine (filled circles; n=17), lidocaine (filled squares; n=5), and procaine (open circles; n=10). Each 5-HT neuron was recorded for at least 3 minutes prior to drug injection to establish a stable baseline firing rate, and then drugs were administered intravenously at 2-minute intervals in a cumulative-dose manner; the percent change from the baseline firing rate was calculated for each dose by using the mean firing rate during the 2-minute period between doses (ordinate). Only one drug was tested per cell and per animal.

of this hypothesis are the observations by Pitts and Marwah (1987a) that pretreatment with reserpine, which depletes neuronal stores of monoamines including 5-HT, significantly attenuates the ability of systemically administered cocaine to suppress midbrain 5-HT activity.

Microiontophoretic drug application techniques have also been utilized in combination with extracellular recording techniques to assess whether cocaine has direct effects on 5-HT cell firing in the DRN (Cunningham and Lakoski 1988); this approach is particularly advantageous as it facilitates the direct investigation of synaptic mechanisms that may mediate the central effects of cocaine. Microiontophoretic application of cocaine produces a current-dependent suppression of spontaneous 5-HT cell firing without concomitant evidence of any apparent local anesthetic effects (figure 3). This depression of neuronal activity by cocaine is characterized by a rate of onset and offset similar to that observed with 5-HT as recorded in the DRN. Cocaine application, at ejection currents that did not initially alter spontaneous baseline firing rates, has been found to potentiate the inhibitory effects of 5-HT when microiontophoretically applied together (Cunningham and Lakoski 1988). In contrast, when applied at currents where no local anesthetic effects were apparent, the microiontophoretic application of procaine consistently failed to inhibit 5-HT neurons.

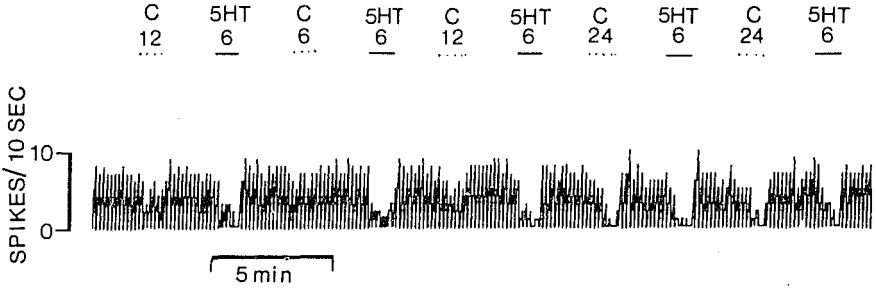

FIGURE 3. *Effects of the microiontophoretic application of cocaine and serotonin (5-HT) on the spontaneous firing rate of 5-HT neurons recorded in the dorsal raphe nucleus*

NOTE: In this frequency histogram, the duration of drug ejection is indicated by the bar above the record; numbers refer to iontophoretic current in nanoamperes used for drug ejection. Cocaine (C; 0.01 M; dots) and 5-HT (5-HT; 0.04 M; solid bars) depressed cell firing at low microiontophoretic currents.

Acute cocaine administration has been characterized to significantly modify the electrophysiologic activity of 5-HT neurons. Acknowledging that repetitive, long-term self-administration of this psychostimulant is not uncommon in the user profile of cocaine abusers

(Fischman 1987), the pharmacological consequences of prolonged exposure to this psychostimulant also merit consideration. Preliminary studies suggest that significant modification of both the cellular pharmacology and physiology of 5-HT neurons is observed in the midbrain raphe nuclei following repeated cocaine exposure (Cunningham et al. 1987a). Clearly, the relevance of this change in 5-HT neuronal function observed with chronic cocaine exposure to psychiatric disturbances observed in abusers remains unknown and merits further investigation. Likewise, an analysis of the ionic mechanisms that may underlie both acute and chronic effects of cocaine on 5-HT function are important areas for future investigations.

While studies utilizing single-unit electrophysiologic approaches to characterize the central effects of cocaine on identified monoamine neurons have only recently been undertaken, several important aspects of cocaine-induced modulation of 5-HT function reveal a similar profile for the monoamines DA and NE. Inhibitory effects of acute systemic cocaine have been identified on the spontaneous activity of DA-containing neurons recorded in the ventral tegmental area (Einhorn et al. 1988; Pitts and Marwah 1987a) and in a postsynaptic terminal region of these cells, the nucleus accumbens (White 1986; White et al. 1987). Analogous to observations regarding 5-HT neuronal function in the DRN, mesoaccumbens, but not mesocortical, projecting DA neurons are inhibited in a rapid, dose-related, and reversible manner (cumulative dose to 50 percent inhibition = 2.2 ± 0.3 mg/kg) following intravenous cocaine administration (Einhorn et al. 1988; Pitts and Marwah 1987a; White 1985); however, complete suppression of baseline firing is not achieved in any of the DA cell groups with systemic cocaine infusion. Similar to responses reported in the DRN, pretreatment with reserpine effectively attenuates cocaine-induced inhibition of DA cell firing recorded in the ventral tegmental area (Einhorn et al. 1988; Pitts and Marwah 1987a); the selective DA antagonist sulpiride blocks this cocaine-mediated suppression of cell firing recorded both *in vivo* and *in vitro* (Brodie and Dunwiddie 1986; Einhorn et al. 1988; White 1986). In studies of the NE-containing cells recorded in the locus coeruleus, the same pattern of cocaine-induced responses emerges as that shown for the 5-HT and DA neuronal systems. Intravenous administration of cocaine rapidly, dose-dependently, and reversibly suppresses NE neurons in this brain region to a maximal 80 percent inhibition of baseline firing rates (Pitts and Marwah 1986a; Pitts and Marwah 1986b; Pitts and Marwah 1987b); likewise, pretreatment with reserpine or administration of the

adrenergic antagonist piperoxane effectively attenuates the stimulant-induced changes in locus coeruleus neuronal activity. The observed differences in the potency of cocaine to suppress the spontaneous activity of monoamine-containing neurons (i.e., 5-HT>NE>DA) suggest possible differential contributions of these neuronal systems to the diverse behavioral effects of cocaine.

Just as cocaine potentiates the inhibitory responses of DRN neurons to 5-HT, similar modulation has also been revealed for DA- and NE-mediated responses. Microiontophoretic application of cocaine significantly potentiates DA-induced depression of cell firing in the ventral tegmental area and nucleus accumbens (Brodie and Dunwiddie 1986; Einhorn and White 1986; Einhorn et al. 1988; White et al. 1987). While the direct microiontophoretic effects of cocaine on identified NE-containing neurons remain, as yet, uncharacterized, pronounced enhancement of NE-mediated responses has been identified utilizing *in vitro* hippocampal (Yasuda et al. 1984) and locus coeruleus (Surprenant and Williams 1987) preparations. These observations, which demonstrate potentiation of 5-HT, DA, and NE responses by cocaine, are in agreement with the neurochemical profile of cocaine as an inhibitor of monoamine reuptake. Clearly, additional studies are needed to further assess the site-specific importance of cocaine-induced modulation of central monoamine function. Single-unit-recording electrophysiologic approaches will continue to provide needed information regarding the central effects of cocaine on these neuronal systems.

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The Effects of Cocaine on Local Cerebral Metabolic Activity

Linda J. Porrino and Conan Kornetsky

INTRODUCTION

The development of the 2-[^{14}C]deoxyglucose (2-DG) method by Louis Sokoloff and his colleagues (1977) has provided a powerful tool with which neuroscientists can investigate the functional events in the brain related to various physiological, pharmacological, and behavioral states. It has been used extensively in neuropharmacology to identify the neural circuits that mediate the effects of a wide variety of pharmacological agents (McCulloch 1982).

The quantitative 2-DG autoradiographic method measures local rates of cerebral glucose utilization in all regions of the central nervous system simultaneously. In the brain, as in all tissues that do physicochemical work, the amount of energy used reflects the amount of work done. Glucose utilization as a measure of energy use under normal physiological conditions (in the brain, glucose is virtually the exclusive substrate for energy metabolism) is coupled to functional activity. Because of this close relationship between energy metabolism and functional activity, it is possible to identify regions of the brain with altered functional activity in various conditions.

The 2-DG method, then, does not measure functional activity directly, but measures glucose utilization, and changes in functional activity are inferred from the changes in glucose utilization. The validity of this assumption has been demonstrated in numerous experiments in which evoked changes in the functional activity of a variety of sensory and motor pathways have been shown by the 2-DG method to be associated with corresponding changes in rates of glucose utilization in the components of the activated or inhibited system (Sokoloff 1982).

Although a number of energy-requiring processes such as synthesis of neurotransmitters and amino acids contribute to the basal rates of glucose utilization, the alterations in glucose utilization evoked by a pharmacological, physiological, or behavioral manipulation are thought to result mainly from increases or decreases in electrical activity, i.e., spike frequency in the central nervous system. These changes in electrical activity in turn produce corresponding increases or decreases in the activity of Na^+, K^+-ATPase, the energy-consuming enzyme involved in the restoration of neuronal ionic gradients to their resting state. Experiments with deoxyglucose have shown that the coupling of glucose utilization to functional activity is dependent on Na^+, K^+-ATPase activity, in that ouabain, an ATPase inhibitor, can block the increases in glucose utilization that accompany electrical stimulation (Mata et al. 1980). Another significant issue in the interpretation of changes in glucose utilization is the site of these changes. A number of studies (Schwartz et al. 1979; Kadekaro et al. 1985) have now clearly demonstrated that functional activation of glucose utilization occurs mainly in nerve terminals rather than in cell bodies. This means that alterations in energy metabolism in a specific brain area are due to changes in the afferent inputs to that structure and not in the cell bodies contained within the structure.

To measure rates of local cerebral glucose utilization, a radioactively labeled analog of glucose, 2-DG, is used, which, like glucose, is transported into cerebral tissue and phosphorylated by hexokinase but, unlike glucose, is trapped within cells. It is this trapping that allows quantitative autoradiography to be used to measure actual rates of glucose utilization in individual brain regions. Calculations are made from the levels of glucose and 2-DG in plasma measured during the experimental period and from the concentration of radioactivity in the tissue measured autoradiographically. The details of the experimental procedures and an extensive discussion of the theoretical basis and mathematical derivation of the 2-DG method is beyond the scope of this chapter, but for further discussion see Sokoloff et al. (1977) and Sokoloff and Porrino (1986).

The most significant advantage of the 2-DG method is that it affords a means to measure quantitatively a dynamic biochemical process in conscious, freely moving animals whose behavior is unrestricted. Furthermore, the 2-DG method makes use of autoradiography, which permits both high spatial resolution so that small anatomical regions and subregions can be identified, and the entire central nervous system can be examined simultaneously. There are, however, several important limitations of the method. First, it is not possible to

identify the specific neurotransmitters, neuromodulators, or receptor types that are responsible for changes in brain electrical activity. The 2-DG method measures only glucose flux. Second, it is not possible to distinguish between inhibitory or excitatory processes since both are energy-requiring. Third, the 2-DG method is unable to differentiate between direct and indirect effects of given stimulus. An entire pathway or circuit may be metabolically activated even though the direct action of the stimulus may occur only at the origin of the pathway. Although this may be a limitation in some instances, as in the case of determining the primary site of action of a drug, it is an advantage when the goal is to identify the neural circuitry that mediates a specific behavioral response.

EFFECTS OF COCAINE ON LOCAL CEREBRAL METABOLIC ACTIVITY

The first report characterizing the functional consequences of cocaine administration in the brain was that by London and her colleagues (1986). In this experiment, three doses of cocaine, 1, 10, and 30 mg/kg injected intraperitoneally, were tested in partially restrained rats. Restricted changes in rates of brain energy metabolism were observed even at the highest dose studied. Effects occurred mainly in the extrapyramidal motor system, generally considered to be important for the production of stereotyped behavior, which was elicited at this dose. An interesting finding in this study was the decreased glucose utilization in the lateral habenula at the 10 and 30 mg/kg doses. Energy metabolism in the lateral habenula is decreased following the administration of other psychostimulants such as amphetamine (Wechsler et al. 1979; Porrino et al. 1984) and dopaminergic agonists such as apomorphine (McCulloch et al. 1982). In addition to the habenula, the pattern of metabolic changes in other brain areas that resulted from cocaine administration in this study was generally similar to those patterns observed with stereotypy-inducing doses of other direct and indirect dopaminergic agonists.

We took a somewhat different experimental approach in our studies of the effects of cocaine on cerebral metabolic activity (Porrino et al. 1988). In designing our experiments, two specific factors were considered. First, the intravenous (IV) route of administration was chosen because the bioavailability that results from the intravenous administration best approximates the bioavailability that results from the routes of administration most often used in humans (Jones 1977). Rates of local cerebral glucose utilization were measured in rats following IV administration of three doses of cocaine (0.5, 1.0, and

5.0 mg/kg). The second factor was the behavioral response to IV cocaine administration. We wanted to relate the alterations in functional activity inferred from changes in glucose utilization to increases in locomotor activity elicited by cocaine. Measurements of locomotor activity were made in freely moving rats during the period of incorporation of the radiolabeled tracer 2-DG. The time of maximal behavioral response to cocaine was matched to the time of maximal incorporation to insure the greatest sensitivity of the method.

We found a highly selective pattern of distribution of alterations in cerebral energy metabolism at the two lowest doses of cocaine tested, 0.5 and 1.0 mg/kg (figure 1). Significant increments in glucose utilization were seen only in the medial prefrontal cortex and the nucleus accumbens. At 1.0 mg/kg, glucose utilization was also increased in the substantia nigra reticulata and decreased in the lateral habenula. Metabolic activity in the rest of the 38 structures examined was not significantly affected by cocaine administration. Behaviorally, these doses produced small, nonsignificant increases in locomotor activity as compared with vehicle controls.

The selectivity of effects at low doses was in striking contrast to the effects at the highest dose tested. Following the IV administration of 5.0 mg/kg, widespread elevations in glucose utilization were present throughout the brain, and these were most prominent in the extrapyramidal motor system (figure 1). Additionally, glucose use was increased in a number of limbic structures including the hippocampus and olfactory tubercle. In all, significant alterations in cerebral energy metabolism were found in 20 of the 38 structures examined. Behaviorally, animals were significantly more active following the administration of the 5.0 mg/kg dose of cocaine than after either vehicle or low-dose cocaine administration.

The significant correlations between locomotor activity and rates of glucose utilization that were found in portions of the extrapyramidal motor system, including the globus pallidus and substantia nigra reticulata, suggest that the changes in functional activity in these areas are a reflection of the alterations in motor function elicited by cocaine administration. In contrast, the pattern of alterations in local cerebral energy metabolism in the medial prefrontal cortex and nucleus accumbens are more in concert with the role that these portions of the mesocorticolimbic dopaminergic system are thought to have in the mediation of cocaine's reinforcing effects than its effects on locomotion. The significance of the medial prefrontal cortex and

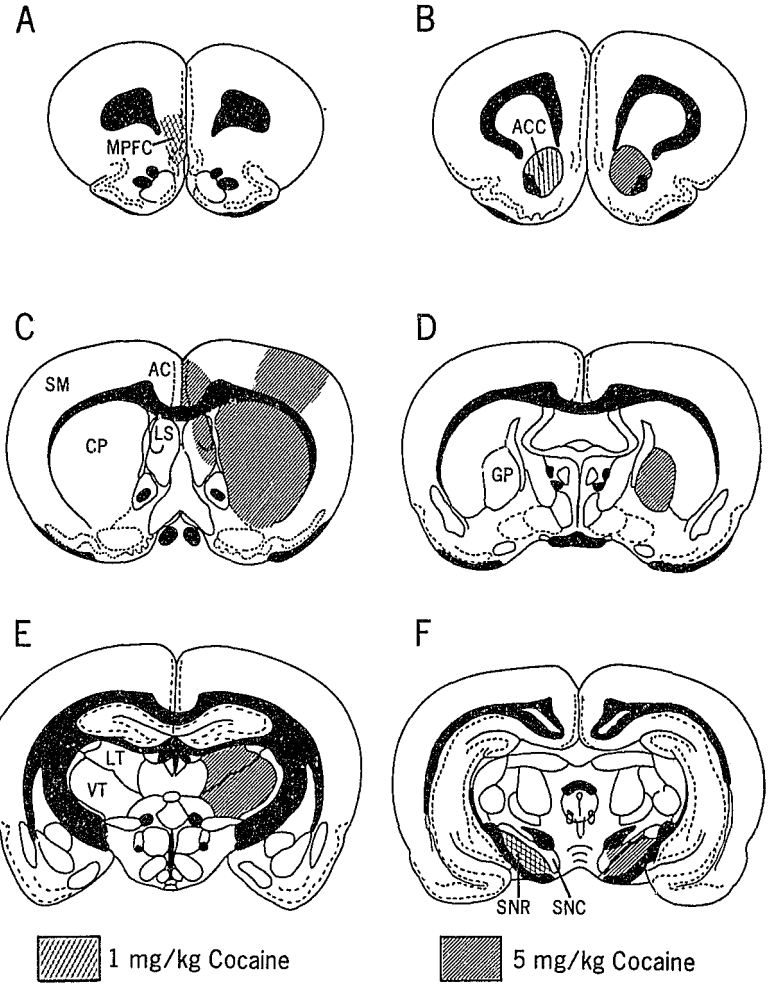

FIGURE 1. *Diagrams illustrating the distribution of alterations in local cerebral glucose utilization associated with the administration of 1.0 mg/kg and 5.0 mg/kg IV, as depicted in six frontal planes (A-F)*

KEY: AC, Anterior cingulate cortex; ACC, nucleus accumbens; CP, caudate-putamen; GP, globus pallidus; LS, lateral septum; LT, lateral thalamus; MPFC, medial prefrontal cortex; SM, somatomotor cortex; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; VT, ventral thalamus.

SOURCE: Adapted from Paxinos and Watson (1982), Copyright 1982, Academic Press, Australia, Ltd.

nucleus accumbens has been demonstrated in a number of studies. Rats, for example, will self-administer cocaine directly into the prefrontal cortex (Goeders and Smith 1983; Goeders, this volume), and lesions of the nucleus accumbens can disrupt responding for the intravenous self-administration of cocaine (Roberts et al. 1977; Roberts et al. 1980; Koob and Hubner, this volume). The fact that the selective metabolic effects of cocaine in these areas occurred only at doses that are known to be reliably self-administered intravenously by rats is further support for the nucleus accumbens and medial prefrontal cortex as neural substrates underlying cocaine reinforcement.

The differences in the distribution of changes in functional activity found in the present study and those reported earlier by London and her colleagues (1986) were perplexing. A number of factors including rat strain, use of restrained vs. freely moving preparations, and the route of administration may account for the discrepancies. We tested one of these possibilities, route of administration, by comparing the effects on local cerebral glucose utilization of an intraperitoneal dose (10 mg/kg) of cocaine to those of an intravenous dose (1.0 mg/kg), both of which produced similar increases in locomotor activity. As in previous studies, rates of glucose utilization did not differ from control values in most of the structures examined. Rates were similarly altered in the substantia nigra reticulata (figure 2) and the lateral habenula following either type of cocaine administration. Intravenously administered cocaine, however, resulted in significant changes in rates of glucose use in the nucleus accumbens (figure 2) and the medial prefrontal cortex. These differences in the distribution of alterations in local cerebral glucose utilization are interesting in light of recent findings by Spyraki and her coworkers (Nomikos and Spyraki 1988; Spyraki et al. 1987), who have shown that route of administration is a significant variable in the establishment of a conditioned place preference in rats. Brain concentrations and the rate at which these concentrations are achieved vary with the route of administration and, therefore, may in turn be significant factors in determining the local metabolic effects as well as the behavioral consequences of cocaine administration (Balster, this volume).

METABOLIC MAPPING OF THE EFFECTS OF COCAINE ON BRAIN STIMULATION REWARD

It has been suggested that abused drugs such as cocaine achieve their reinforcing properties by activation of reward systems in the brain (Kornetsky 1985). One common approach to the study of these

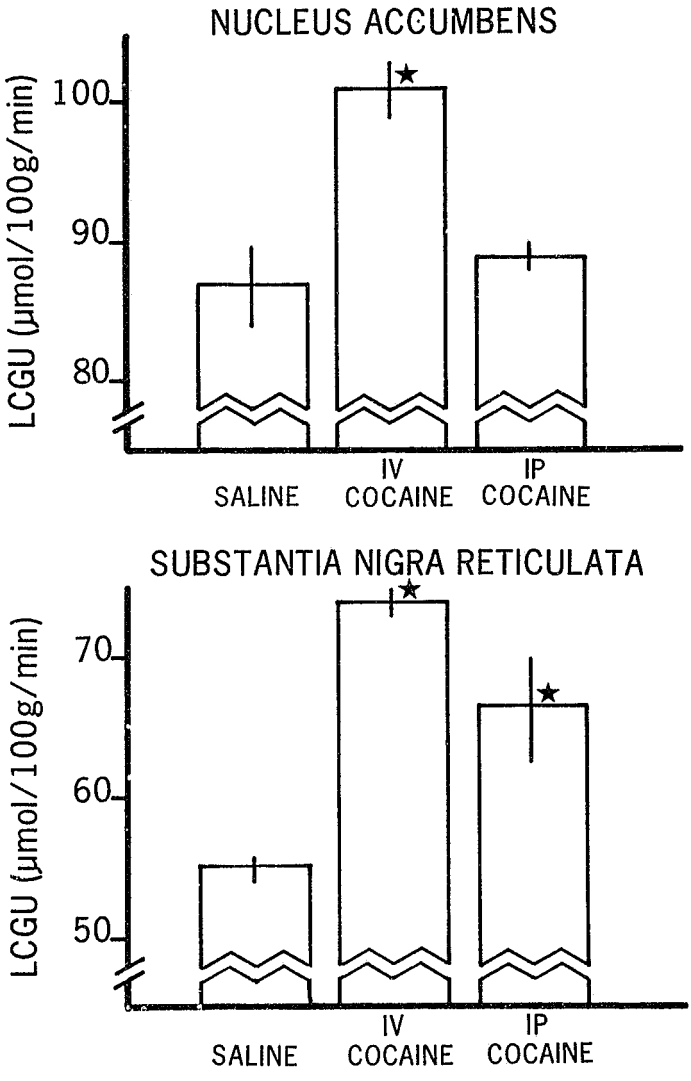

FIGURE 2. *A comparison of the effects of saline vehicle, and acute intravenous (1 mg/kg), and intraperitoneal (10 mg/kg) administration of cocaine on local cerebral glucose utilization (LCGU) in the nucleus accumbens and substantia nigra pars reticulata in rats*

*p<0.05, significantly different from saline vehicle control, Bonferroni t-statistic.

NOTE: Values represent means and standard errors of rates of LCGU (µmol/100 g/min) in groups of six, four, and four rats, respectively.

systems is through brain stimulation reward, a phenomenon first demonstrated by Olds and Milner (1954), who showed that an animal will work in order to obtain electrical stimulation to certain brain sites.

Cocaine has been shown both to increase rates of responding for a constant level of intracranial stimulation (Crow 1970) and to lower the threshold for brain stimulation reward (Esposito et al. 1978). The specific neural circuitry involved in mediating these effects, however, has not yet been identified. To address this question, we have used metabolic mapping with the 2-DG method to identify those areas functionally involved in the interaction of cocaine and brain stimulation reward (Bain et al. 1987). Two groups of animals were tested: (1) animals self-stimulating to the medial forebrain bundle; and (2) animals self-stimulating to the medial forebrain bundle that received 10 mg/kg cocaine intraperitoneally. This dose and regimen were chosen because previous work has clearly demonstrated its effectiveness in enhancing brain stimulation reward (Esposito et al. 1978). Both groups of animals receiving electrical brain stimulation responded on the average at identical rates at similar current intensity levels. In these preliminary experiments, these data were compared to a group of animals with electrodes implanted in the medial forebrain bundle but not stimulated during the experiment (Porrino, unpublished observations).

Although the experiment is not complete, an interesting picture has emerged. In most areas, rates of local cerebral glucose utilization were the same in the two groups of animals responding to brain stimulation reward. Increases in metabolic activity as compared to rates in unstimulated rats were found throughout the rostral and caudal extent of the medial forebrain bundle and in areas such as the nucleus accumbens, prefrontal cortex, and amygdala. Marked bilateral increases in rates of glucose utilization were found in the olfactory tubercle and decreases were found in the ventral tegmental area of cocaine-treated animals as compared with rates in animals receiving electrical stimulation alone. Metabolic activity in these areas has been reported not to be altered following intraperitoneal cocaine administration without brain stimulation (London et al. 1986). It is worth noting that the effects of the interaction of cocaine and brain stimulation reward on cerebral metabolism in the olfactory tubercle are quite similar to those reported with amphetamine and brain stimulation (Seeger et al. 1984). Finally, these data suggest that cocaine enhances brain stimulation reward by acting on circuits different from those that mediate its effects on locomotor activity.

The behavioral state of the animal during cocaine administration, then, is a significant factor in determining the anatomical distribution of local cerebral metabolic effects.

COMPARISON WITH OTHER PSYCHOMOTOR STIMULANTS

Psychostimulants can be divided into two separate drug classes on the basis of their actions at dopaminergic nerve terminals (McMillen 1983; Scheel-Kruger 1971; Shore 1976). Drugs in the amphetamine class of stimulants act via stimulation of release of newly synthesized dopamine from an alpha-methyl-para-tyrosine sensitive pool (Chiueh and Moore 1975b; Scheel-Kruger 1971). In contrast, drugs in the nonamphetamine class of stimulants (e.g., cocaine or methylphenidate) release dopamine from reserpine-sensitive vesicular stores (Chiueh and Moore 1975a; Scheel-Kruger 1971). Although there are differences in the biochemical actions of these drugs, there are strong similarities in their behavioral effects (e.g., reinforcing properties, locomotor-activating properties, and stereotypy-eliciting properties), all of which are thought to be mediated through their effects on dopaminergic systems (Creese and Iverson 1975; DeWit and Wise 1977; Roberts et al. 1975).

There have been a number of studies examining the effects of psychostimulants other than cocaine on brain glucose utilization. The dose-dependent nature of cocaine's effects on local cerebral energy metabolism is clearly analogous to the effects of other psychostimulants on functional activity. Low doses of either amphetamine (Porrino et al. 1984) or methylphenidate (Porrino and Lucignani 1987), for example, result in selective increases in glucose utilization in the nucleus accumbens (figure 3). Again, as with cocaine, there appears to be a greater sensitivity of anatomical portions of the mesocorticolimbic dopaminergic system, as compared with components of other dopaminergic systems, to the actions of psychostimulants.

At higher doses, parallel effects on glucose utilization and behavior also occur. Both amphetamine (Wechsler et al. 1979; Porrino et al. 1984) and methylphenidate (Bell et al. 1982; Porrino and Lucignani 1987) produce functional changes in many of the components of the extrapyramidal system similar to those produced by cocaine (Porrino et al. 1988; London et al. 1986). At these doses, all three psychostimulants elicit stereotypic behavior patterns that may include repetitive sniffing, grooming, rearing, circling, and/or gnawing (Fog 1969).

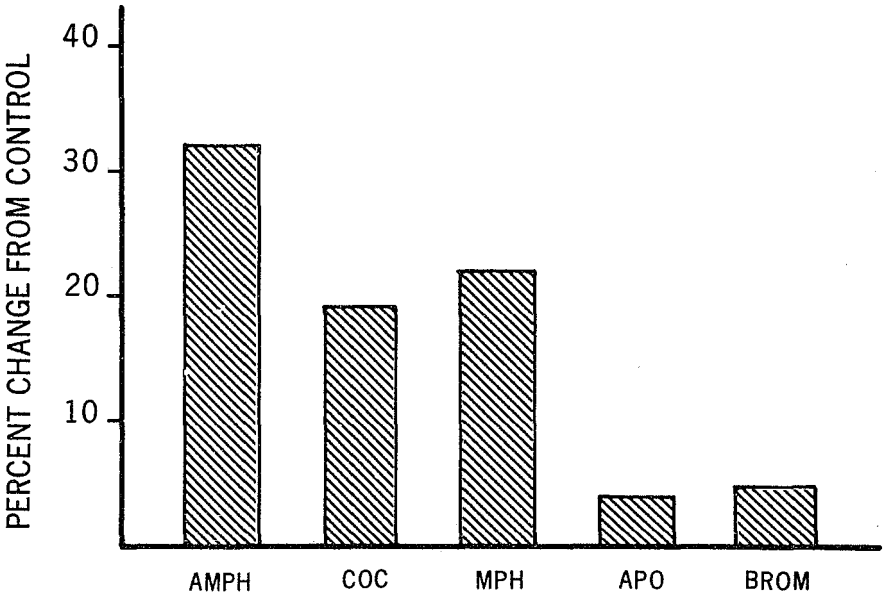

FIGURE 3. *Comparison of the alterations in local cerebral glucose utilization accompanying the administration of selected psychostimulants and dopamine receptor agonist drugs*

NOTE: Values are percent change from control. AMPH, amphetamine (0.5 mg/kg) (Porrino et al. 1984); COC, cocaine (1 mg/kg) (Porrino et al. in press); MPH, methylphenidate (2.5 mg/kg) (Porrino and Lucignani 1987); APO, apomorphine (0.5 mg/kg) (McCulloch et al. 1982; Porrino, unpublished observations); BROM, bromocriptine (20 mg/kg) (Pizzolato et al. 1985).

Although this dose-dependent pattern of effects within the mesocorticolimbic and nigrostriatal dopaminergic systems seems to be typical of psychostimulants, such a pattern is not characteristic of other dopaminergic drugs. For example, apomorphine, a direct dopamine receptor agonist, does not significantly alter glucose utilization in the mesocorticolimbic system. It is without effect on such areas as the nucleus accumbens (figure 3) and prefrontal cortex (McCulloch et al. 1982). This pattern is similar to that obtained following the administration of bromocriptine, another dopamine receptor agonist (Pizzolato et al. 1985) (figure 3). It appears that

psychostimulants have a unique metabolic action in the mesocorticolimbic dopaminergic system not observed with other dopaminergic agonist drugs.

CONCLUSIONS

The quantitative 2-DG method maps changes in functional activity based on experimentally measured alterations in local rates of cerebral glucose utilization and has proved useful in pharmacological applications. The series of studies reviewed here emphasize the complexity of the neural circuitry that mediates the effects of cocaine in the central nervous system. In particular, they show how variables such as dose, route of administration, and behavioral state can determine the neurochemical response to cocaine administration. Finally, cerebral metabolic studies of psychostimulants and other drugs acting at dopaminergic synapses demonstrate a unique sensitivity of the dopaminergic mesocorticolimbic system to the effects of psychostimulants. In summary, the 2-DG method can provide a comprehensive picture of the functional involvement of various brain areas in response to pharmacological agents such as cocaine and may be able to furnish information about the neural circuits involved in cocaine use and abuse.

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Behavioral Studies of the Reinforcing Properties of Cocaine

Chris E. Johanson

INTRODUCTION

This chapter will describe the behavioral characteristics of cocaine self-administration and highlight the procedures that have been used in experimental studies to measure its reinforcing efficacy. Since this monograph is devoted in large measure to reviewing the status of our understanding of the neuroanatomical and neurochemical bases of cocaine's properties that contribute to its abuse by humans, it is necessary to clarify the intent of including a review of behavioral studies on cocaine's reinforcing efficacy.

Like all psychoactive drugs, cocaine has a complex, multiple set of pharmacological properties mediated by a number of neurochemicals with sites of action throughout the central nervous system. Not all of these properties are related to the reinforcing efficacy of the drug; in addition, it is likely that the mediation of cocaine's reinforcing properties is due to the interaction between multiple systems. In studying the molecular basis of cocaine's reinforcing effects, the influence of this complexity on behavioral indices of reinforcing efficacy is often ignored. Including a review of behavioral studies demonstrating that cocaine self-administration is not solely determined by cocaine's reinforcing effects and that reinforcing properties can be influenced by a range of behavioral variables is a reminder to neuroscientists that any molecular mechanism proposed to underlie cocaine's addictive properties must be able to account for these complexities. This chapter is also intended to acquaint neuroscientists who are studying the molecular basis of cocaine's reinforcing properties with the most sophisticated approaches for measuring these effects, so that they can design the most definitive studies.

ESTABLISHMENT OF COCAINE AS A REINFORCER

One of the definitions of a positive reinforcer is the ability to maintain responding leading to its presentation. The positive reinforcing properties of cocaine have been investigated since the early 1960s, using drug self-administration techniques. Pickens and Thompson (1968) showed that rats equipped with an intravenous catheter would press a lever if that response was followed by an intravenous injection of cocaine. In a series of manipulations, these investigators carefully demonstrated that lever pressing was a function of contingent cocaine injection. For instance, when cocaine was injected intermittently by the experimenter instead of the animal, the rats did not continue to lever press. When saline was substituted for cocaine or when only a light change followed each lever press, responding declined. Finally, when two levers were available, the rat responded on the lever that produced cocaine even when the experimenter switched operative levers.

In another early self-administration study (Wilson et al. 1971), rhesus monkeys were given 4 hours of daily access to cocaine during which each lever press resulted in a drug injection. Interestingly, the monkeys regulated their drug intake to a remarkable degree. After training, they showed stability in their daily intake of cocaine over periods of months. There were no indications of changes in sensitivity to cocaine's reinforcing effects as would be indicated by an increase (tolerance) or a decrease (supersensitivity) in its rate of self-administration. These investigators also demonstrated the constancy of cocaine intake by changing the dose injected after each lever press. As dose per injection was increased, the number of injections taken by the animals decreased, resulting in an almost constant intake in drug regardless of the dose per injection.

Another type of regulation is also evident in the pattern of cocaine self-administration. As shown in figure 1, infusions of cocaine are equally spaced across the experimental session almost as if the drug were being injected under the control of a clock. Stability of intake within sessions is not a characteristic shared by many other drug reinforcers, as shown for pentobarbital in figure 1. Even a drug such as amphetamine, which shares many pharmacological properties with cocaine, shows variation in intake; within sessions, amphetamine is taken in bursts of injections with long pauses between these bursts (Balster and Schuster 1973a). On the other hand, a variety of other drugs that function as positive reinforcers and also have some properties in common with cocaine, including procaine (Johanson 1980),

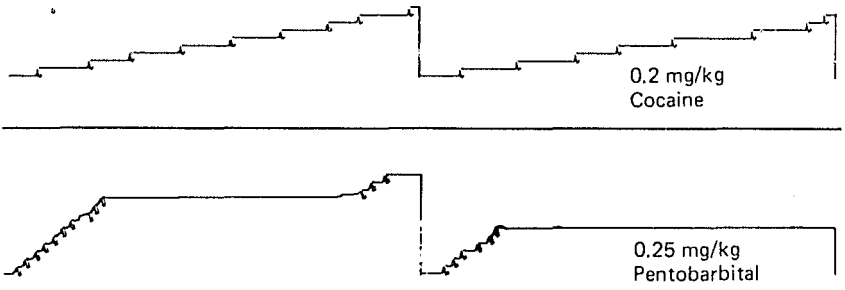

FIGURE 1. *Representative cumulative response records of fixed-ratio 10 performance maintained by 0.2 mg/kg cocaine (top panel) and 0.25 mg/kg pentobarbital (bottom panel) in a rhesus monkey*

NOTE: Ordinate: cumulative responses. Abscissa: time. Each downward deflection of the response pen indicates a 10-second injection. The response pen resets every 30 minutes.

cathinone (Johanson and Schuster 1981b), and propylbutyldopamine (Woolverton et al. 1984), also show within-session regularity in intake. The mechanisms resulting in this type of regularity are not currently understood. It is unlikely, however, that it is completely attributable to any single set of neurochemical events.

What must also be explained by a complete description of the molecular actions of cocaine is the disappearance of controlled intake across sessions when access to cocaine is no longer limited to a few hours each day. In a study by Johanson et al. (1976), untrained rhesus monkeys were exposed to continuous around-the-clock access to one of a variety of psychomotor stimulant drugs. Two monkeys given access to 0.2 mg/kg cocaine began taking drug the very first day of its availability, and immediately intake became erratic and excessive, resulting in severe toxicity that led to death. Similar results were noted with other psychomotor stimulants tested, including amphetamine, methamphetamine, and diethylpropion. Therefore, it appears that, where there are no outside restraints on the availability of psychomotor stimulant drugs, rhesus monkeys will suddenly increase their drug-taking behavior to the point of severe toxicity. In contrast, the intake of cocaine under conditions of limited access is surprisingly regulated. The mechanism underlying this loss of control is not understood, but it is possible that it is due to psychomotor

stimulant drugs' ability to produce stereotypic behavior, which may include the drug-taking response.

REINFORCING PROPERTIES OF COCAINE ACROSS CONDITIONS

The results of the early studies of Pickens and Thompson (1968) and Wilson et al. (1971) have been replicated in a number of ways. What is impressive is the generality of the phenomenon. For instance, cocaine is self-administered by every species of animal tested, including rats (Pickens and Thompson 1968), squirrel monkeys (Goldberg 1973; Katz 1979), rhesus monkeys (Woods and Schuster 1968), pigtail macaques (Young and Woods 1980), baboons (Griffiths et al. 1975), dogs (Risner and Jones 1975), and humans (Fischman 1984). This concordance across species, both in terms of the ability of cocaine to function as a reinforcer as well as the characteristics of the maintained responding (Griffiths et al. 1980; Johanson and Schuster 1981a), is one type of evidence of the robustness of cocaine's ability to function as a reinforcer. This also implies that similar molecular substrates are shared across species and that it is unlikely that cocaine abuse in humans is due to some specific pathological condition.

A second type of evidence that cocaine is a robust reinforcer is that it maintains responding regardless of its route of delivery. Although the IV route has been used most commonly in experimental studies, cocaine also maintains responding when delivered intragastrically (Woolverton and Schuster 1983), by chewing or smoking (Siegel et al. 1976), and intramuscularly (Goldberg et al. 1976).

Cocaine self-administration occurs not only with a variety of species using several routes of administration but also under a variety of environmental circumstances. In the terminology of behavior analysis, this can be translated into schedule contingencies, i.e., the rule that governs the relationship between behavior and the injection of the reinforcer. The nature of that rule has overwhelming influence on the reinforcing properties of cocaine, as evidenced by changes in the shape and position of its dose-response function. Such an influence is yet another of the behavioral characteristics of cocaine that require elucidation by any proposed molecular mechanism.

In the simplest schedule, continuous reinforcement, every response is followed by a reinforcer. This schedule was used by both Pickens and Thompson (1968) and Wilson et al. (1971). However, the

relationship between responding and reinforcer presentation may be made more complex. A response may be reinforced on the basis of the number of responses emitted since the termination of the previous reinforcer presentation (a ratio schedule), or on the basis of the time elapsed since the last reinforcer presentation (an interval schedule). Many studies have shown that cocaine maintains responding under ratio schedules (Balster and Schuster 1973a). The pattern of responding is characterized by an initial pause followed by a high terminal rate of responding (figure 1). Although this pattern of ratio responding is similar to that maintained by other events, such as food and water, the rates of responding typically found in drug self-administration studies have been low compared to rates maintained by food, and increases in dose per injection further decrease rates, i.e., the relationship between dose and rate of responding is inverse. These low rates are most likely due to the dual actions of the drug, as shown in figure 2. On the one hand, cocaine serves as a reinforcer that increases rate of responding, but on the other hand, the drug has the ability to disrupt ongoing behavior temporarily, and thus has a rate-decreasing effect. As dose is increased, the latter effects predominate, and responding becomes suppressed. Since increased responding under ratio schedules results in increased rates of drug intake, the problem is particularly striking under this schedule. Therefore, using ratio schedules to determine the potency of cocaine *as a reinforcer* may be inadequate because the dependent variable

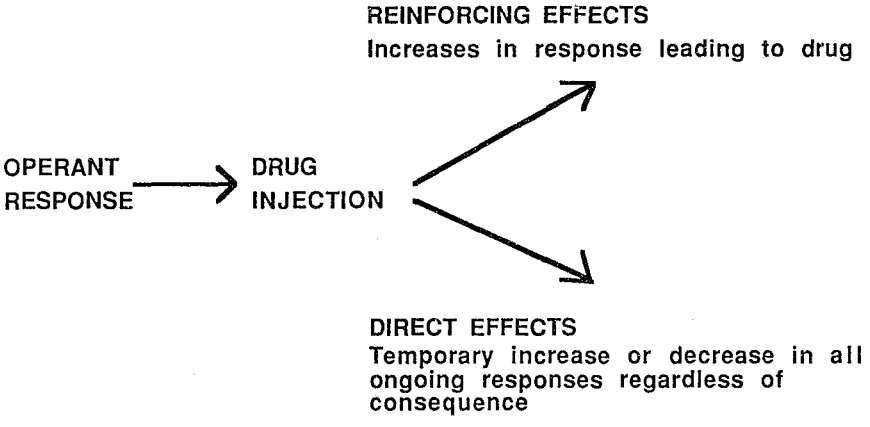

FIGURE 2. *Two types of effects that a drug injection contingent on an operant response can exert*

itself, namely rate of responding, is not determined solely by reinforcing properties. In fact, at the highest doses, which may have the maximum reinforcing effect, responding is predominantly influenced by cocaine's nonspecific rate-decreasing effects and is often suppressed. This problem must be taken into consideration in interpreting studies designed to determine the underlying molecular mechanism of any specific behavioral effect such as reinforcing properties.

In an attempt to avoid a confounded measure of reinforcing properties, other schedules have been used in cocaine self-administration studies. These studies are also important in terms of further demonstrating the generality of cocaine's reinforcing properties. For instance, cocaine has been demonstrated to maintain responding under interval schedules. An important feature of interval schedules is that rates of responding can change considerably without affecting rate of reinforcement. One of the first studies using an interval schedule of cocaine injections in monkeys was conducted by Balster and Schuster (1973b). Responding was maintained under a fixed-interval, 9-minute schedule of cocaine injection in one component and food delivery in the other. In addition, there was a 15-minute timeout following the presentation of each reinforcer. Responding was well maintained, and the pattern of gradually accelerated responding over the interval with cocaine was similar to that maintained by food. As dose per injection increased, rate of responding increased, i.e., the dose-response function was direct. However, in a study by Johanson (1982) using a fixed-interval, 5-minute schedule of cocaine injection without an intervening timeout period, the shape of the dose-response function was no longer a direct one but was inverted U-shaped. That is, rate of responding initially increased as dose per injection was increased, but rate then decreased with further increases in dose. This difference was most likely due to the more frequent injections in the Johanson study. Despite the powerful nature of the contingencies governing reinforcer presentation controlling responding, the nonspecific rate-modifying actions of cocaine also exerted an influence. Once again, this complicates assessments of the molecular mechanisms underlying the reinforcing properties of cocaine that rely on the ability to compare potencies across measures.

Several studies with cocaine have used second-order schedules as a way of minimizing the direct effects of cocaine, to get a less confounded estimate of the drug's reinforcing actions. In this type of schedule, responding specified by one particular schedule is treated as a unitary response that is itself reinforced by another schedule (Kelleher 1966). The responding treated as a unitary response can

also be followed by the presentation of a brief stimulus paired with the presentation of the reinforcer. Goldberg (1973), using squirrel monkeys, studied responding maintained by cocaine under a fixed-ratio (FR) 30 schedule of stimulus presentation (2-second yellow light), which itself was maintained under a fixed-interval (FI) 5-minute schedule of cocaine injection. This schedule is designated a second-order FI 5-minute (FR 30:S). Under this schedule, rates of responding for cocaine were extremely high and similar to responding maintained by other events, such as food, under an identical schedule. Several additional studies have been conducted with this schedule, using both squirrel and rhesus monkeys. Typical responding has been maintained under FI (FR) schedules by IV and IM cocaine as well as with FR schedules of FI components (Goldberg et al. 1981; Kelleher and Goldberg 1977; Goldberg and Kelleher 1977; Goldberg et al. 1975; Goldberg et al. 1976).

Kelleher and Goldberg (1977) demonstrated the importance of the brief stimuli in maintaining responding under second-order schedules. When these stimuli were removed following the FR components but the drug was still injected, rate declined and patterning was disrupted. If both drug injections and the brief stimuli were removed, responding declined even further. However, when the brief stimuli were then reinstated without the drug, responding increased. Similar results have been found in other studies including one with IM cocaine (Katz 1979). The fact that both the drug and the stimuli are determinants of the rate of responding may explain the results when dose is manipulated with this schedule. Although there is some tendency for rate to increase with increases in dose, in general, dose-response functions are flat relative to those generated by other schedules. Therefore, if rate of responding reflects reinforcing properties, the strength of cocaine's ability to control responding does not seem to change with its magnitude under these schedule conditions. This is not the case under other schedules (Balster and Schuster 1973b; Iglauer and Woods 1974; Johanson and Schuster 1975), and, under second-order schedules with different parameters, the shape of the dose-response function is an inverted U-shape (Johanson 1982).

In summary, the studies reviewed in this section demonstrate that cocaine self-administration occurs under a variety of experimental circumstances and is not restricted to a narrow range of conditions. It is clear that persistent and excessive drug-seeking behavior is determined by an interaction between the drug's schedule of presentation and its specific pharmacological properties. The shape and

position of cocaine's dose-response function can change dramatically as a function of the parameters of the experimental conditions. To some extent, this dynamic quality is due to the multiple actions of cocaine (e.g., rate-suppressing effects, stereotypic effects), so it is important in studies that are focused on elucidating the underlying mechanism of cocaine's reinforcing properties to bear in mind possible confounds of the behavioral measure. In addition, however, it is possible that the reinforcing properties of cocaine as well as other drugs are altered by a variety of factors including behavioral ones. One example of a behavioral effect that has a profound influence on cocaine self-administration is level of food deprivation. It has been shown (de la Garza et al. 1981) that the rate of responding increases under both a ratio and an interval schedule of cocaine injection when rhesus monkeys are food restricted. When molecular mechanisms of action of cocaine's reinforcing effects are proposed, it will be essential to determine how variables that alter the behavioral measure of cocaine's reinforcement alter the molecular substrate.

REINFORCING EFFICACY ASSESSED USING NONRATE MEASURES

Because of the difficulty of using indices that can be influenced by effects of cocaine other than reinforcing properties, the development of additional approaches has been vigorously pursued. Two other procedures that have been used to compare different doses of cocaine (in the absence, it is hoped, of any confounding influence) are choice paradigms and concurrent schedules. In these procedures, responding on different levers is maintained by different doses, and the primary dependent variable is the relative frequency of occurrence of the alternative responses. The actual rate at which either of these responses is made, which can be dramatically influenced by level of drug intake, is irrelevant and does not contribute to an assessment of reinforcing properties. These procedures have also been used with other reinforcers, such as food and intracranial stimulation, and have been found to be sensitive to differences in reinforcer magnitude.

With concurrent schedules, responding is maintained by two or more simultaneously operating schedules. In a study by Iglauer and Woods (1974), responding was maintained in rhesus monkeys under a concurrent two-lever variable interval schedule of cocaine injections with a 5-minute timeout following each injection. In this study, relative reinforcing efficacy was evaluated by comparing relative response frequencies on the two levers. A standard dose of cocaine (0.05 or 0.1 mg/kg) was available under a variable interval 1-minute schedule

on one of two levers; the dose available under an identical schedule on the second lever (variable-dose lever) was varied to include both higher and lower doses of cocaine. The proportion of responses occurring on the variable-dose lever increased as the dose available on that lever increased; in all cases, the larger of the two doses presented for comparison was preferred.

The second procedure designed to compare reinforcing properties involves the use of discrete choice trials. In a study by Johanson and Schuster (1975), rhesus monkeys were given an opportunity to choose between two doses of cocaine, and injections were followed by a 15-minute timeout period. The number of trials during which one option rather than the other was selected was counted and used as the measure of reinforcing properties. As in the Iglauer and Woods (1974) study, actual rate of responding did not influence the measure of reinforcing properties, and again it was found that higher doses of cocaine were preferred to lower doses. Similar results have been found by Brady and Griffiths in baboons (1977).

The results that have been obtained with both the concurrent schedule and the choice paradigm have been encouraging. The assumption in studies of drug self-administration is that reinforcing strength or efficacy ought to increase with dose. As indicated in the prior section, dose-response relationships with cocaine are rarely direct, and it has been assumed that this was due to rate-modifying effects of cocaine's action unrelated to reinforcing properties. To the extent that the influence of these other effects has been eliminated in studies using procedures that do not utilize rate as a measure of reinforcing efficacy, these procedures are likely to be useful in the elucidation of the molecular mechanisms underlying the reinforcing properties of cocaine.

REINFORCING PROPERTIES ASSESSED BY RESISTANCE TO PERTURBATION

In addition to the use of nonrate procedures, other approaches for evaluating the reinforcing efficacy of cocaine and other drug reinforcers have been developed to a limited extent. These approaches have in common the notion that the strength of a reinforcer can be measured in direct proportion to its ability to maintain responding, even when that responding is perturbed by an outside influence. The perturbations that have been used in experimental studies include increasing the work required to obtain the reinforcer (increased

response cost), providing other mutually exclusive reinforcer alternatives, and concurrent punishment.

The influence of response cost on drug self-administration has been evaluated using progressive-ratio schedules. In such a schedule, responding is maintained by a drug under a ratio schedule. After responding is well established, the number of responses required for each drug injection is systematically increased until responding declines to below some criterion, i.e., animals at these high ratios no longer continue to respond in order to get drug. The ratio value that leads to this cessation in responding is called the breaking point. Although responding is maintained under a ratio schedule, the breaking point, not rate of responding, is used as the index of reinforcing efficacy. It does not matter how long an animal takes to complete the ratio (within limits) but simply whether or not it is finished. Using this procedure, Yanagita (1973) demonstrated that breaking point was a direct function of the dose of cocaine, as would be expected. At the highest dose (0.48 mg/kg), animals continued responding even when 6,400 to 12,800 responses had to be made for each drug injection. Johanson (1975) used a combination of the previously described choice procedure and a progressive-ratio schedule. Under the initial condition, animals given a choice between a low and a high dose selected the high dose. Next, the fixed-ratio requirement necessary to produce the preferred dose was systematically increased, while the behavioral requirements for the alternative but less preferred dose of cocaine remained the same. It was reasoned that, although animals prefer higher doses of cocaine over lower doses, if the behavioral requirements for the preferred dose were great enough, the animals would choose the alternative. In addition, the greater the difference between the sizes of the doses of the two alternatives, the greater the increase in ratio necessary to alter preference. In two of the four monkeys tested, the results were as predicted (figure 3). However, for the remaining two monkeys, the high dose continued to be selected even when ratios were above 300 responses per injection. Given the results found by Yanagita (1973), it is likely that higher ratios were required to alter preference in these two monkeys.

Progressive-ratio schedules have also been used to compare the reinforcing properties of cocaine to other drug reinforcers. For instance Yanagita (1973) found that cocaine's breaking point was 2 to 16 times higher than that for methamphetamine and amphetamine. Similar results were found by Bedford et at. (1978). Griffiths and colleagues determined that the breaking point for cocaine was higher

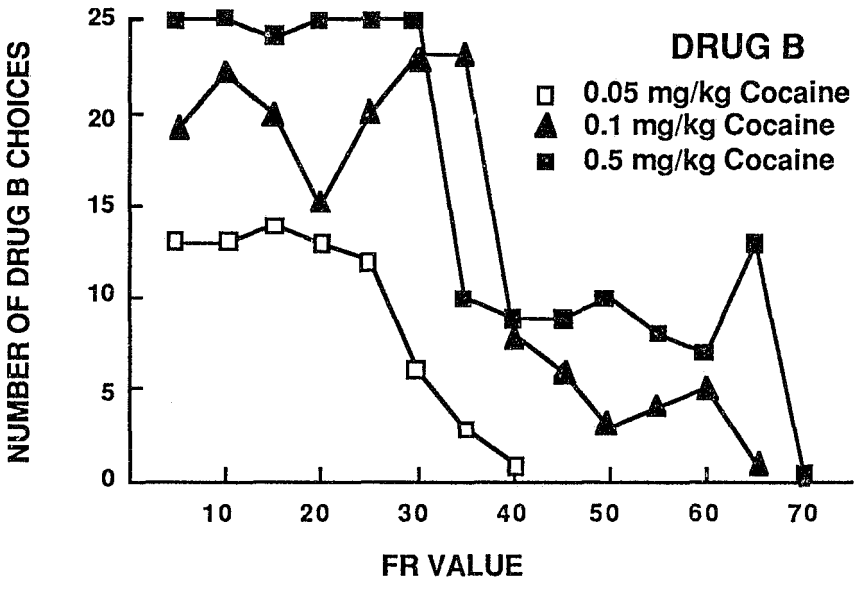

FIGURE 3. *Number of choice trials in which different doses of cocaine (drug B) were chosen over 0.05 mg/kg of cocaine as a function of fixed-ratio requirements for the injection of drug B*

NOTE: The fixed-ratio requirement for the injection of 0.05 mg/kg cocaine was 5. The maximum number of choice trials programmed each session was 25.

than that for other stimulant or anorectic drugs including methylphenidate, diethylpropion, chlorphentermine, and fenfluramine (Griffiths et al. 1975; Griffiths et al. 1978). Studies using dogs have also demonstrated that cocaine sustains responding at higher FR values than *d*-amphetamine, mazindol, fenfluramine (Risner and Silcox 1981), or nicotine (Risner and Goldberg 1983). The important point of these studies is that cocaine is more efficacious than these other drugs as a reinforcer, and the extent of the difference can be expressed numerically by comparing maximum breaking points.

Another type of perturbation is the availability of alternative reinforcers, the selection of which eliminates the opportunity for cocaine self-administration. The choice procedure developed by Johanson and Schuster (1975) has been used in this regard to compare different drugs to cocaine. When differences in potency are taken

into account, cocaine was found to be more efficacious as a reinforcer than methylphenidate (Johanson and Schuster 1975), diethylpropion (Johanson and Schuster 1977a), and procaine (Johanson and Aigner 1981). Interestingly, a choice procedure that compared cocaine to *dl*-cathinone, the active alkaloid of a plant (khat) that is chewed recreationally by inhabitants of Africa and the Middle East, found that the two drugs had similar efficacy (Woolverton and Johanson 1984).

In addition to comparisons between cocaine and other drugs, there have been choice studies utilizing alternative nondrug reinforcers. For instance, monkeys preferred even low doses of cocaine to the opportunity to have visual contact with other monkeys (Woolverton, personal communication). Even more compelling, monkeys given a choice between food and cocaine preferred the latter and, without experimenter intervention, might have starved (Aigner and Balster 1978). As with the progressive-ratio and drug/drug choice studies, these results clearly indicate that cocaine is a powerful reinforcer, i.e., its reinforcing efficacy exceeds that of most other reinforcers.

The third approach to assessing the strength of a reinforcer is to determine its resistance to the effects of punishment. The effects of punishment such as electric shock and time out from positive reinforcement on behavior controlled by a variety of events other than drugs have been studied extensively. The degree of response suppression is dependent upon the intensity of the punishing event and its schedule of presentation, as well as on the time between response and consequence. All else being equal, it would be assumed that the greater the difficulty in decreasing, using punishment, the self-administration of a particular drug, the greater is that drug's reinforcing strength.

The effects of punishment on cocaine self-administration have been demonstrated in several studies. Grove and Schuster (1974) examined the ability of punishment to suppress responding maintained by cocaine injections in monkeys under a FR 1 schedule during daily 3-hour sessions. Punishment was accomplished by delivering a brief electric shock at the onset of each injection. Responding maintained by both 0.1 and 0.2 mg/kg decreased as a function of the intensity of the shock. However, the degree of suppression expressed as a percent of control rates was the same for the two doses. That is, increasing the magnitude of reinforcement did not seem to attenuate the effects of punishment, as might be expected if one assumes that higher doses of cocaine have greater reinforcing efficacy. This finding, however, is difficult to interpret because the baseline rates

of responding maintained by the different doses of cocaine were not the same. Because responding was maintained under a ratio schedule, the rates maintained by the higher dose were lower.

In an attempt to eliminate the problem of rate differences, Johanson (1977) used the discrete trial choice procedure previously described, with added punishment contingencies. Rhesus monkeys were given a choice between two alternatives of IV cocaine. These alternatives were initially equal in dose, but in subsequent comparisons they differed in magnitude. Electric shock was delivered at the onset of the injection of one of the alternatives. When the two doses were equal, the nonshocked alternative was chosen. For some animals, the shocked alternative was preferred even when the dose of this alternative was only twice as high. Other animals continued to select the nonshocked alternative. However, as the dose of the shocked alternative further increased, all animals preferred the higher dose. Figure 4 shows the results from one of these monkeys.

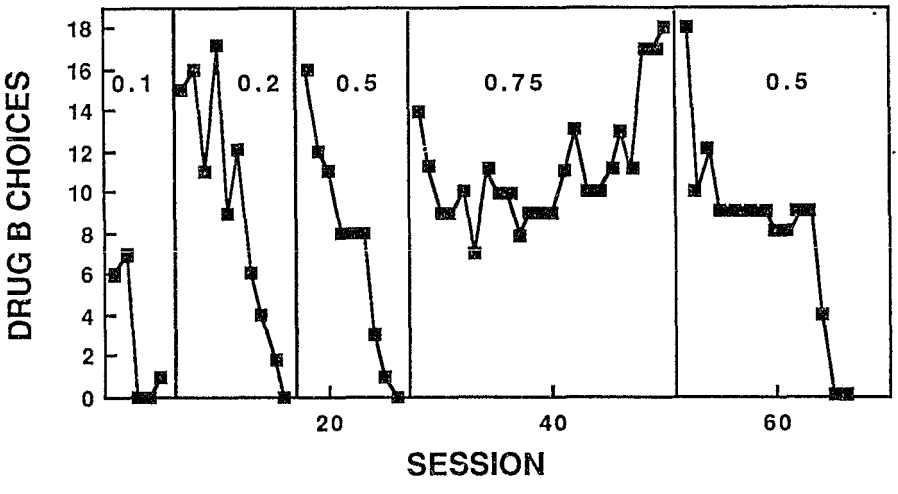

FIGURE 4. *The number of injections of the higher dose (indicated above each panel) selected by an individual monkey during consecutive sessions*

NOTE: Electric shock was delivered at the onset of each injection of this higher dose. The lower dose was 0.1 mg/kg cocaine during the entire session. There was a maximum of 18 choices per session.

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All of the studies reviewed in this section clearly lead to the conclusion that cocaine has strong and robust reinforcing properties, as indicated by its ability to continue to maintain responding even when response cost is high, even at the expense of refusing alternative reinforcers as important as food, and even when self-administering cocaine is punished. All of these approaches can be utilized to compare cocaine's reinforcing efficacy to that of other drugs, but to date not enough data have been generated to indicate unequivocally that any of these approaches will be successful in quantitatively differentiating reinforcing efficacy across drugs. If these approaches are sensitive to differences in reinforcing efficacy across drugs and yield accurate quantitative indices of these differences, these values could be used for correlating with the effects of cocaine on various molecular systems. However, it is important to note that the results of these studies appear to indicate that even drugs with similar pharmacological actions (e.g., cocaine, mazindol, amphetamine, procaine) differ not only in potency but also in efficacy. How drugs with differing efficacy on a behavioral property (reinforcing properties) can be compared in molecular studies that assess differences in potency is not clear.

SUMMARY

In this chapter, the behavioral characteristics of cocaine self-administration were described. Cocaine is a powerful positive reinforcer, and its reinforcing properties have been demonstrated in every species tested, across an enormous range of conditions in experimental studies with animals. To compare the reinforcing efficacy of cocaine with that of other drugs, to determine how these effects might change under different environmental conditions, and, in the present context, to determine their underlying molecular mechanisms require that its reinforcing properties be assessed using indices that are not influenced by the multitude of other pharmacological effects that are produced by cocaine. Many procedures rely on rate of responding or levels of cocaine self-administration as a measure of reinforcing properties, but these may not be adequate. The use of more sophisticated approaches that utilize nonrate measures or an assessment of the resistance of drug-maintained responding to perturbations may enable neuroscientists to design more definitive studies.

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Pharmacological Modifications of Cocaine and Opioid Self-Administration

Gail Winger

INTRODUCTION

Two types of pharmacological treatment of drug abuse have received the most scientific consideration. One is the use of agonists to suppress drug self-administration, the other is the use of antagonists to block drug effects. Agonist treatment approaches for opioid abuse are typified by the use of methadone. Antagonist treatment approaches have more promise under some circumstances, but early attempts to use cyclazocine or naltrexone for opioid abuse were unsuccessful, due primarily to lack of client acceptance of these drugs (Archer 1981; Renault 1981).

Treatment of cocaine abuse with agonists such as methylphenidate has also been attempted, but with little success except in patients with attention deficit disorders (Gawin 1985). To my knowledge, use of dopamine antagonists to treat cocaine abusers has not been attempted, perhaps because these agents can produce serious and permanent motor disorders, unpleasant subjective effects, or increases rather than decreases in cocaine self-administration in experimental animals.

This review discusses some of the animal literature on modification of opioid agonists or opioid antagonists and compares these data to the effects of stimulants or dopamine antagonists on behavior maintained by cocaine administration. Since our understanding of receptor interactions in the opioid system is much clearer than is our understanding of the interactions between cocaine and the dopamine receptor system, the opioid system may serve as a model of agonist-antagonist interactions in the context of drug self-administration.

In using either agonist or antagonist administration to decrease drug self-administration in an animal model, it is important to produce a specific decrease in behavior maintained by the drug. Behaviors maintained by other reinforcers should be left relatively intact by these manipulations, since disruption of behavior in general would be unacceptable in a treatment program for human drug abusers. There are several ways in which specificity of action can be assessed experimentally. One approach is to compare data obtained in animals whose behavior is maintained by food with data from a separate group of animals whose responding is maintained by drug. If similar rates and patterns of behavior develop in both groups of subjects, comparison of the effect of drug administration on behavior maintained by different reinforcers is quite straightforward. Alternatively, food and drug can be used to maintain responding in a single group of subjects. Food and drug may be concurrently available or each reinforcer may be sequentially available, usually by using a multiple schedule of food- and drug-reinforced responding. Thus, the effects of pretreatment drug, either agonist or antagonist, can be seen on behavior maintained by both reinforcers.

AGONIST ADMINISTRATION

The effects of opioid agonists on opioid-reinforced responding have been studied using concurrent or multiple schedules of food- or drug-reinforced responding. John Carney, in his 1976 doctoral thesis (Carney, unpublished), used a multiple schedule of reinforcement, in which signalled periods of food availability alternated with differentially signalled periods of codeine availability. There were five food components and four codeine components in a session, presented in alternation. Each component lasted until 15 reinforcers (either food or codeine) had been earned on a fixed-ratio-30 (FR 30) schedule, or until 15 minutes had passed. The dose of codeine used to maintain behavior in the periods of drug availability was 0.1 mg/kg/injection, a dose that was high enough to result in decrements in codeine-reinforced responding that increased progressively throughout the session. Interestingly, even when rates of codeine-maintained responding were near zero, when the light signalling food availability was illuminated, the monkeys immediately resumed food-reinforced responding at a high rate. Thus, codeine appeared to be selectively reducing rates of responding maintained by the drug itself.

Administration of a number of opioid agonists, including morphine, etorphine, and levorphanol, produced decrements in codeine-maintained responding at doses that produced little change in

food-maintained responding. Although this suggested a selective action of opioid agonists on opioid-maintained responding, the rates of responding maintained by food were considerably higher than rates of responding maintained by codeine. If the opioid agonists were simply suppressing low rates of responding selectively, the apparent differential effect of drug due to reinforcer type could be spurious. Carney did a rate-dependent analysis of the effects of the agonists on different rates of responding maintained by codeine and food and concluded that rate differences alone could not account for the differing effects of opioids on codeine- and food-maintained responding.

Even more convincing data on a selective effect of opioid agonists on opioid-maintained responding were reported by Griffiths et al. (1976). These investigators provided baboons with a choice between food and heroin during eight daily trials. The dose of heroin was selected so that the animals typically selected drug on three of the trials, and food on the remaining five trials. Administration of 8.3 mg/kg/day methadone produced a decrement in choice of heroin trials and a corresponding increase in selection of food.

With the exception of the study reported by Mello et al. (1983) in which methadone did not affect self-administration of dilaudid, the data are consistent with respect to the effects of opioid agonists on opioid self-administration. There appears to be a selective suppression of opioid-maintained responding by opioid agonists that is not necessarily accompanied by a similar suppression in food-maintained responding. Thus, the evidence we have concerning the effectiveness of methadone in suppressing opioid administration in human opioid abusers is, for the most part, supported by the data obtained in animals.

There are fewer published studies of the effects of cocaine agonists on cocaine self-administration. Herling et al. (1979) evaluated the effects of cocaine, amphetamine, and pentobarbital on behavior maintained by cocaine in one group of monkeys and by food in a second group. The schedule of reinforcement was the same in both groups. It was a second-order schedule in which a brief light stimulus was presented following every 10th response, and the 10th response following a fixed interval of 5 minutes was reinforced with delivery of food or cocaine, accompanied by the light stimulus. Rates of responding maintained by food or cocaine were variable from monkey to monkey, but overlapped to a considerable degree. The effects of each of the three pretreatment drugs were independent of

the reinforcer delivered. Rates of responding maintained by food or cocaine were suppressed to an equal degree by similar doses of either cocaine, d-amphetamine, or pentobarbital.

Although these data suggest that the rate-decreasing effects of cocaine or amphetamine on cocaine-maintained responding may not be selective for cocaine, there are other data that indicate that dopamine agonists may have a selective effect on cocaine-maintained responding. Woolverton and Kleven (1987) presented such evidence using behavior maintained under a multiple schedule of food- and cocaine-reinforced responding. The schedule was similar to that used by Carney (unpublished) in which periods of food availability alternated with periods of drug availability. In Woolverton's study, however, a single period of cocaine-reinforced responding was placed between two periods of food-reinforced responding. Rates of responding under all conditions were high, and there was little difference in rates maintained by food and those maintained by cocaine.

Under these conditions, constant infusions of the dopamine agonist bromocriptine produced a selective decrement in rates of responding maintained by cocaine, while leaving rates maintained by food relatively intact. This very provocative report was tempered by the additional information that the chronic infusion of bromocriptine produced toxic effects of increased activity, stereotyped behavior, and preconvulsive signs in some of the monkeys, and the infusions could not be continued.

The primary difference between the effects of opioid agonists on opioid self-administration and those of dopamine agonists on cocaine self-administration is that the opioid agonists produce selective decreases in opioid-maintained responding in the absence of clear toxic effects on the animal. The dopamine agonist bromocriptine was also able to suppress selectively cocaine-maintained responding but produced marked toxic effects. Clearly, the data with both types of drug reinforcers are very limited, and further study remains to be done before it is clear what, if any, differences exist in these two systems. Among other things, the current data should be replicated, the effects of other dopamine agonists must be evaluated, and comparisons should be made between the effects of acute and chronic administration of the agonists in question.

In designing an agonist that might be capable of suppressing behavior maintained by cocaine, it is important to keep in mind the aspects of methadone that may be related to its success in the treatment of

opioid abuse. It has a sustained effect following a single administration, it has a slow onset of action that may decrease the likelihood of its having strong reinforcing effects in its own right, it has few toxic effects on chronic administration, and it can be given for sustained periods of time without endangering the individual. None of these aspects is true of dopamine agonists such as bromocriptine. Although it may be possible to identify or synthesize dopamine agonists with long durations of action and/or slow onsets of action, it may not be possible to identify a dopamine agonist that either does not have or does not develop toxic effects with chronic administration. Tolerance to the effects of dopamine agonists has not been clearly defined. In the case of some stimulants such as *d*-amphetamine, which may have a dopamine agonist component of action, sensitivity to some actions has been described (Robinson and Becker 1986). Although it is much too soon to tell whether dopamine agonists will have some use in the treatment of cocaine abuse, it appears to this author that the problem of toxic effects on chronic administration will be the most difficult to overcome.

ANTAGONIST ADMINISTRATION: SELF-ADMINISTRATION OF SINGLE DOSES

The effects of antagonist administration on opioid self-administration have been described in a relatively small number of experiments. Using the schedule of alternating food- and 0.1 mg/kg/injection codeine-maintained responding described above, Carney (unpublished) showed that naltrexone produced marked alterations in codeine-maintained responding at doses that did not markedly alter food-maintained responding. The codeine-selective nature of the effects of naltrexone was most obvious at a dose of 0.32 mg/kg given prior to an experimental session. Rates of behavior maintained by codeine were almost completely eliminated, whereas rates of behavior maintained by food were relatively unaltered. Lower doses of naltrexone, 0.003 and 0.03 mg/kg, produced marked increases in rates of responding maintained by codeine and increases in the amount of drug self-administered. These doses also had little effect on behavior maintained by food.

In support of Carney's findings of increases in opioid self-administration following administration of an antagonist, Griffiths et al. (1976) reported a dose-related increase in choice of heroin over food in baboons chronically infused with naltrexone.

The increase in opioid-maintained responding following administration of opiate antagonists is much like the effects of dopamine antagonists on cocaine-maintained responding. A recent review of the literature on the effects of antagonists on cocaine self-administration and discrimination (Woods et al. 1987) indicated that, when dopamine antagonists were given prior to opportunities for cocaine self-administration, rates or amounts of cocaine self-administration were increased. The dopamine antagonists used included haloperidol in both rats and monkeys; chlorpromazine in both rats and monkeys; pimozide, sulpiride, thioridazine, and flupenthixol in rats; and pimozide in both dogs and squirrel monkeys.

Although these studies of cocaine and codeine self-administration indicated that antagonists produced increases in behavior maintained by these drugs, in most cases only a single dose of self-administered drug was used. It is difficult to make generalizations about the effects of antagonists on the reinforcing effects of agonists under these circumstances. The general pattern of drug self-administration under typical fixed-ratio schedules of reinforcement is an inverted U-shape (e.g., Young and Woods 1980; Winger and Woods 1985). If antagonists act to shift this curve to the right in a parallel fashion, then doses on the ascending limb of the inverted U-shaped curve would maintain lower rates of responding following antagonist administration, and doses on the descending limb would maintain higher rates of responding following antagonist administration. We have argued previously (Woods et al. 1987) that it is the ascending limb that reflects most accurately the reinforcing function of cocaine and other drugs, with the descending limb being modulated predominantly by the rate-decreasing effects of the drug. Thus, it is when the ascending limb is shifted by pretreatment with potential antagonists that a specific effect on reinforcing function can be indicated most clearly. In studies where rates of cocaine- or opioid-maintained responding were increased by pretreatment with an antagonist, the dose of the reinforcing drug was almost always sufficiently high to be on the descending limb of a dose-effect curve, had one been obtained. It is thus not surprising to find consistent increases in behavior following administration of antagonists. Unfortunately, these experiments did not reveal much about the effects of antagonist administration on the reinforcing effects of the agonists, as would have been shown if lower doses of the agonists had been evaluated.

ANTAGONIST ADMINISTRATION: MULTIPLE SELF-ADMINISTERED DOSES

The effects of infusions of naltrexone on self-administration of a range of doses of morphine was evaluated by Harrigan and Downs (1978). In the absence of naltrexone, behavior maintained by morphine occurred at a variety of rates, depending on the dose of morphine available. An inverted U-shaped curve relating dose to rates of responding was observed. This curve was shifted to the right with a slightly decreased maximum by an infusion of 1 μg/kg/hr of naltrexone. An infusion rate of 20 μg/kg/hr of naltrexone produced an overall decrement in responding maintained by all tested doses of morphine.

A similar pattern of nonsurmountable antagonism of opioid self-administration by naltrexone was observed by Herling (1981). He evaluated the effects of naltrexone on rates of food- and codeine-maintained responding, using a schedule of alternating periods of food and codeine availability. A dose of naltrexone of 0.003 mg/kg produced a slight shift to the right in both the ascending and descending limbs of the codeine dose-rate function. A dose of 0.03 mg/kg naltrexone produced a further shift to the right, as would be expected if naltrexone were antagonizing both the rate-reinforcing and rate-decreasing effects of codeine. However, when the dose of 0.03 mg/kg naltrexone was administered on two further occasions, rates of codeine-maintained responding were decreased and flattened rather than being shifted in a parallel fashion to the right. Importantly, these doses of naltrexone had little effect on food-maintained responding. In fact, administration of naltrexone was able to antagonize the suppression of food-maintained responding that developed following self-administration of fairly high doses of codeine.

As an aside, it may be that the nonsurmountable effect of naltrexone on opioid-maintained responding is due primarily to the nature of the naltrexone-agonist interaction. Bertalmio and Woods (1987) have shown parallel shifts to the right in behavior maintained by alfentanil by administration of 0.1 and 1.0 mg/kg of the opiate antagonist quadazocine. No tendency for a downward shift in the alfentanil dose-rate function was observed over frequent administration of different doses of quadazocine.

Using different doses of self-administered agonists to evaluate the effects of antagonists has also been shown to be important with the

dopamine antagonist-cocaine interaction. One of the very few exceptions to the finding of increases in cocaine self-administration following administration of a dopamine antagonist was that reported by Herling and Woods (1980). In this study, behavior was maintained by either food in one group of animals or cocaine in another group of animals. Reinforcement was delivered on a second-order schedule for both food and cocaine; rates, as described earlier for this type of schedule, were variable among animals, regardless of the reinforcer used. Administration of chlorpromazine produced only decreases in rates of behavior maintained by food. In the case of cocaine, however, the effects of chlorpromazine were highly dependent on the dose of cocaine use to maintain the behavior. The effects of chlorpromazine on behavior maintained by low doses of cocaine were identical to the effects of chlorpromazine on behavior maintained by food: rates were decreased across a range of chlorpromazine doses. With higher doses of cocaine maintaining behavior, however, the effects of chlorpromazine were to increase rates of responding maintained by cocaine. Interestingly, under this schedule of reinforcement, increasing doses of cocaine did not produce consistent changes in rates of responding, although two of the four monkeys showed increases in rates followed by decreases in rates, as the dose of cocaine was increased. The authors interpreted their findings as indicating a mutually antagonistic interaction between cocaine and chlorpromazine. Whereas chlorpromazine was able to antagonize the rate-maintaining and rate-decreasing effects of cocaine, cocaine (when delivered in sufficiently high doses), was also able to reverse the rate-decreasing effects of chlorpromazine.

Although the typical inverted U-shaped function relating dose of cocaine to rate of responding was not shown by all animals in the preceding study, such a curve was obtained (Herling, unpublished) in a study evaluating the reinforcing effects of food and several doses of cocaine using a fixed-ratio schedule of reinforcement. In this series of experiments, periods of food-maintained responding alternated with periods of cocaine-maintained responding. As shown in figure 1, the effects of various doses of chlorpromazine on rates of responding maintained by increasing doses of cocaine was to shift, in a fairly selective way, the ascending limb of the cocaine dose-rate curve. Interestingly, the rate-decreasing effects of cocaine, as indicated by the descending limb of the curve, were not modified nearly as much. This is in contrast to virtually all of the previous reports of a rate-increasing effect of chlorpromazine on cocaine self-administration. The reason for the lack of effect of chlorpromazine on the descending limb of the curve in this particular study is not

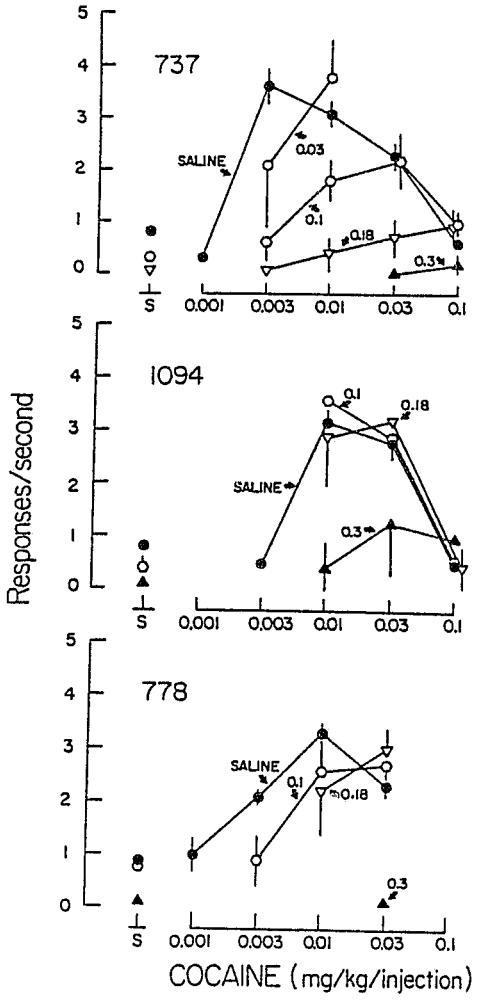

FIGURE 1. *Effects of chlorpromazine on behavior maintained by various doses of cocaine*

NOTE: Abscissae: Dose of intravenous cocaine (mg/kg/inj) available on an FR 30 schedule of reinforcement. Ordinates: Rates of responding in responses per second. Closed circles are the rates of responding maintained by cocaine following pretreatment with saline. The other symbols are rates maintained following pretreatment with 0.03 mg/kg chlorpromazine (open circles), 0.1 mg/kg chlorpromazine (hexagons), 0.18 mg/kg chlorpromazine (inverted triangles), and 0.3 mg/kg chlorpromazine (closed triangles). The numbers 737, 1094, and 778 refer to individual monkeys.

SOURCE: Herling 1980, Copyright 1980, Seymore Herling.

understood. These data indicate, however, that chlorpromazine, administered under these experimental conditions, could modify the reinforcing effects of cocaine, as indicated by shifts to the right in the ascending limb of the cocaine dose-effect curve.

The doses of chlorpromazine that were effective in decreasing cocaine-maintained responding also produced decreases in food-maintained responding in the same monkey. Thus, under the particular experimental circumstances described, chlorpromazine was effective in modifying the reinforcing effects of cocaine, but only at doses that also decreased rates of food-maintained responding. This contrasts with data obtained under very similar circumstances in which, as described above, naltrexone suppressed codeine-maintained responding but left food-reinforced responding unaffected.

CONCLUSIONS

The contrast between the effects of dopamine agonists on cocaine self-administration and the effects of opioid agonists on opioid self-administration is in keeping with the differences seen in studies with antagonists. In the case of dopamine agonist and antagonist administration, it appears difficult to find doses that alter cocaine self-administration without producing unacceptable effects on other behavior. The agonists appear to produce toxic effects in the form of increases in stereotyped behavior, and the antagonists produce decreases in food-maintained responding. Neither of these kinds of effects has been reported in carefully controlled studies within the opioid system. Thus, whereas the dopamine agonist bromocriptine appears to be able to produce selective alterations in cocaine-maintained responding, and the dopamine antagonist chlorpromazine appears able to produce selective effects on the reinforcing effects of cocaine, neither of these drugs represents a satisfactory approach to treatment of cocaine abuse.

These experiments represent only the first steps in evaluation of pharmacological modification of cocaine self-administration. There are some reports that antidepressant drugs, e.g., desipramine (Gawin and Kleber 1984) and trazodone (Small and Purcell 1985) appear to have some use in the clinic in reducing cocaine abuse. These drugs must be evaluated in experimental animals using self-administration studies to help us determine what types of experimental models and situations are sensitive to the same types of manipulations that appear effective in man. With this information, it may be possible

to determine the mechanism of action of these antidepressant medications, and perhaps understand more about how cocaine produces its reinforcing effects.

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Reinforcement Pathways for Cocaine

George F. Koob and Carol B. Hubner

INTRODUCTION

Cocaine as a Psychomotor Stimulant

Cocaine produces effects similar to many other drugs classified as psychomotor stimulants. For example, cocaine possesses both anorectic and psychostimulant properties and, as a result, has for centuries been used to suppress hunger and fatigue (Angrist and Sudilovsky 1976). Clinical studies assessing its subjective effects have demonstrated that cocaine has stimulant actions and produces euphoria (Fischman et al. 1983). In animals, cocaine acutely increases motor activity (Groppetti et al. 1973), decreases food intake (Groppetti et al. 1973), has stimulantlike actions on operant behavior (Spealman et al. 1977), enhances conditioned responding (Spealman et al. 1977), decreases threshold for reinforcing brain stimulation (Kornetsky and Esposito 1981), and readily acts as a reinforcer for drug self-administration (Pickens and Thompson 1968). Cocaine shares most of the psychomotor stimulant effects of low doses of amphetamine except that it is shorter acting and less potent (Simon 1973). At higher doses, cocaine acutely produces the intense stereotypy associated with amphetamine or apomorphine but is much less potent (Randrup and Munkvad 1970) and may be less effective (Simon 1973).

Cocaine is also a local anesthetic and has convulsant properties (Matsuzaki 1978). With chronic administration, these convulsant properties appear to become sensitized as do the motor effects of cocaine (Post et al. 1976). This sensitization may be analogous to "kindling" associated with repeated electrical brain stimulation.

Neuropharmacology of Cocaine

The local anesthetic properties of cocaine are not thought to be important for the production of its acute psychological effects, since similar subjective effects cannot be observed with other local anesthetics (Fischman et al. 1983). It is now well established that cocaine has important effects on monoamine metabolism. Cocaine blocks norepinephrine and dopamine reuptake and increases catecholamine turnover (Groppetti et al. 1973). Cocaine also blocks the uptake of tryptophan, thereby presumably reducing serotonin synthesis (Knapp and Mandell 1972) and turnover (Friedman et al. 1975).

Impetus for a critical role for dopamine in the psychomotor stimulant effects of cocaine was the observation that the locomotor activation produced by cocaine could be blocked by 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (Kelly and Iversen 1976). In that study, the lesion effect was thought to be largely due to dopamine depletion, since norepinephrine forebrain depletions were minimized by pretreatment with desmethylimipramine. Furthermore, similar lesions block the locomotor stimulant effects of *d*-amphetamine but not of caffeine or scopolamine (Joyce and Koob 1981), heroin, or corticotropin-releasing factor (Swerdlow and Koob 1985; Vaccarino et al. 1986).

COCAINE AND REWARD THRESHOLD

Substrates of Reward—Intracranial Self-Stimulation

The discovery that electrical stimulation of particular areas of the brain could reinforce behavior provided a unique means to assess the motivation to respond to hedonic stimuli. Indeed, the discovery of intracranial self-stimulation (ICSS) by Olds and Milner (1954) suggested a "short-circuiting" of the reinforcement process. ICSS is typically obtained from most regions of the limbic system. Although electrodes placed in a midbrain-forebrain system (the medial forebrain bundle), which courses through the lateral hypothalamus, produce the highest rates of responding, ICSS has also been produced in regions as far removed from the classical limbic system as the cerebellum and nucleus solitarius.

The potency of ICSS as a reinforcer led to its rapid use as a tool to measure activity in the brain "reward" systems, but also led to many problems of measurement and interpretation. One of the earliest measures of brain stimulation reward was the absolute rate of

responding. Here an experiment consisted of placing an animal in the test situation for a specified period of time using a specified supra-threshold current intensity. Testing was continued until response rates had stabilized from session to session. Generally, this rate of responding was taken to reflect the reinforcing value of the stimulus delivered. Any treatment that increased the rate of responding was thought to reflect a facilitation of reward or a lowering of threshold for reward. This principle, where the actual value of the reinforcer would be directly related to the output of the organism to obtain said reinforcer, has a theoretical basis in a motivational theory (Hernstein 1971).

One means of evaluating threshold for brain stimulation, or by extrapolation measuring "hedonia," has been to establish within each test session the point at which the rat fails to consistently respond. Not unlike psychophysical tests, rats are systematically subjected to an ascending or descending series of rate-intensity functions. What typically results is a sigmoidal function whose slope increases the closer one approaches the low threshold areas of midbrain/lateral hypothalamus (figure 1). Shifts of this function to the right or left without changes in the maximal rate of responding presumably reflect changes in threshold for rewarding brain stimulation.

More recently, Kornetsky and associates have exploited the high-incentive, low-drive properties of ICSS to develop a rate-independent measure of reward (Kornetsky and Esposito 1981). Rate measures per se can lead to misleading results when attempting to measure reinforcement threshold. For example, in an early study, Valenstein (1964) showed that rats allowed a choice between brain stimulation in two different sites showed a preference for the site that maintained the lower rate of responding. Thus, rate-independent threshold measures for a given reinforcer may be a more valid measure of the motivation to respond for a reinforcer.

For the Kornetsky threshold procedure, trials begin with the delivery of a noncontingent 0.5-second stimulus. If the animal responds by turning a wheel manipulandum one-quarter turn within 7.5 seconds of the onset of the "priming stimulus," a second stimulus is delivered that is identical to the first noncontingent stimulus. The intensity of the stimulus (value of the reinforcer) is varied according to a psychophysical procedure where stimuli are presented in an alternating descending and ascending series with five trials presented at each intensity level. The threshold value for each series is

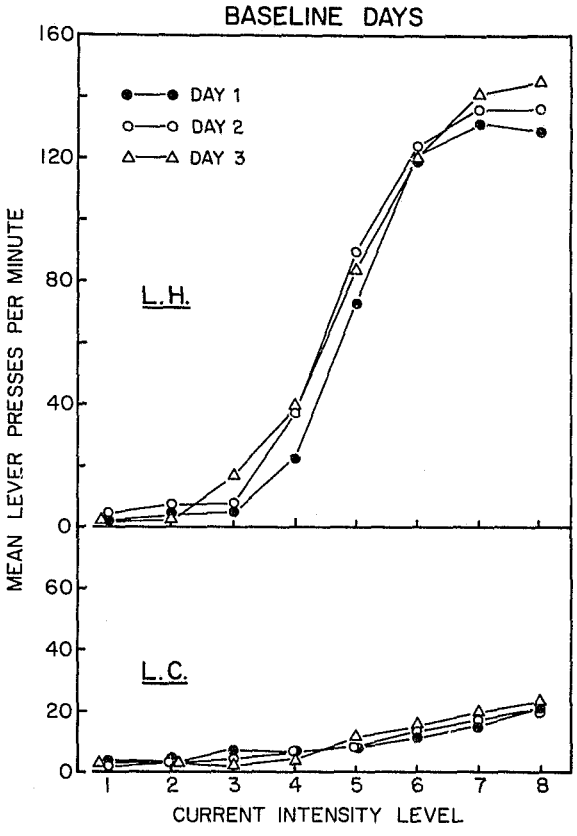

FIGURE 1. *Rate vs. intensity function for intracranial self-stimulation in rats*

NOTE: Eight rats had electrodes placed in the lateral hypothalamus (LH), and seven rats had electrodes aimed at the locus coeruleus (LC). Each curve represents one session with a descending sequence. Currents ranged from 5 to 40μA (60 Herz AC RMS) for the LH and 15 to 50μA for the LC.

defined as the midpoint in current intensity between the level at which the animal made three or more correct responses out of five stimulus presentations, and the level where fewer than three correct responses were made (Marcus and Kornetsky 1974). This "rate-free" measure of reward has proved sensitive to pharmacological manipulation, which is consistent with the hypothesis that it reliably measures reward threshold.

Cocaine and Intracranial Self-Stimulation

In general, drugs that increase the availability of catecholamines at the synapse facilitate ICSS, and those agents that deplete the brain of catecholamines or block catecholamine transmission inhibit ICSS. For example, it is well documented that psychomotor stimulants such as amphetamine will further decrease responding when responding has been suppressed by a punisher (Geller and Seifter 1960). These effects have been described as "a sensitization of the neural systems that mediate the processing of rewards" (Stein 1964). This facilitation of reward by amphetamine is evident in studies using ICSS as the reinforcer (Cassens and Mills 1973; Goodall and Carey 1975; Koob et al. 1977; Phillips and Fibiger 1973; Stein 1964; Stein and Ray 1960; Wauquier and Niemegeers 1974a). Although most studies have employed a lever press response as the operant and have shown that lever-pressing rates for ICSS increase with injection of amphetamine, other measures of threshold have produced similar consistent changes in ICSS threshold (Kornetsky and Esposito 1979). Cocaine also facilitates responding for brain stimulation reward (Crow 1970; Wauquier and Niemegeers 1974b).

While little work has explored site-specific effects for the facilitation of brain stimulation by drugs, one study using a choice procedure and continuous access to brain stimulation from three separate electrodes in the same animals showed that rats preferred to self-stimulate in the ventral tegmental area at moderate and high doses of *d*-amphetamine (Koob et al. 1977). In that study, rats given a choice of self-stimulating in the septal area, anterior lateral hypothalamus, or posterior lateral hypothalamus chose the posterior lateral hypothalamus under the influence of *d*-amphetamine. When given a choice of self-stimulating in the posterior lateral hypothalamus, the ventral tegmental area, or the region of the locus coeruleus, the rats chose the ventral tegmental area under the influence of *d*-amphetamine. The authors suggested that this pattern of preference choices under the influence of the drug parallelled the nondrug thresholds. Anatomically, this preference pattern also parallels the course of the mesolimbic dopamine system.

Amphetamine also decreases ICSS threshold in self-titration procedures where the current delivered decreases systematically with each lever press, but the animal can reset the current back to its original level by pressing another lever (Stein and Ray 1960; Zarevics and Setler 1979). Functions relating rate of responding to current level,

i.e., rate/intensity (R/I) functions, shift to the left with administration of psychostimulants including cocaine (Steiner and Stokeley 1973; Phillips and LePiane 1986), as can be seen in figure 2. Other tests have been developed to minimize the contribution of nonspecific activating effects of psychomotor stimulants on the facilitation of ICSS. Amphetamine and cocaine also enhance reward in these "rate-free" measures of ICSS threshold (Liebman and Butcher 1974; Esposito et al. 1978; Kornetsky and Esposito 1979). Using the threshold procedure, Kornetsky and Esposito (1981) demonstrated that cocaine lowered the reward threshold at doses that actually increased the detection threshold (figure 3). For detection threshold measures, the initial noncontingent stimulus varied in intensity (at subreward levels), while the second or response-contingent stimulus was held constant at a rewarding intensity (above threshold) in order to maintain responding. Thus, the initial stimulus acted as a discriminative stimulus indicating the availability of brain stimulation as a reward (Kornetsky and Esposito 1981). This suggests that cocaine was having an effect on reward threshold that was different from its effect on the ability of the animals to make a psychophysical discrimination.

The mechanism of action for this threshold-lowering action of cocaine is not well understood, but it is sensitive to moderate to high doses of opioid antagonists. Naloxone injected intraperitoneally at doses of 2 to 8 mg/kg attenuated the threshold-lowering actions of cocaine (10 to 15 mg/kg, intraperitoneally), with the most effective dose being 4 mg/kg of naloxone (Bain and Kornetsky 1987). Similar effects of naloxone have been observed with other psychomotor stimulant drugs. Amphetamine lowers brain stimulation threshold, and this effect is also reversed by naloxone (Esposito et al. 1980). These results suggest some form of functional catecholamine/opioid interaction in cocaine reward. However, the specific nature of this interaction needs to be elucidated, particularly given the lack of effect of opioid antagonists on cocaine self-administration.

INTRAVENOUS COCAINE SELF-ADMINISTRATION: NEURAL SUBSTRATES

Pharmacological Studies

Catecholamines have been strongly implicated in the reinforcing properties of psychomotor stimulants (Pickens et al. 1978), and, more specifically, the reinforcing properties of psychomotor stimulants have been linked to the activation of central dopamine neurons and their

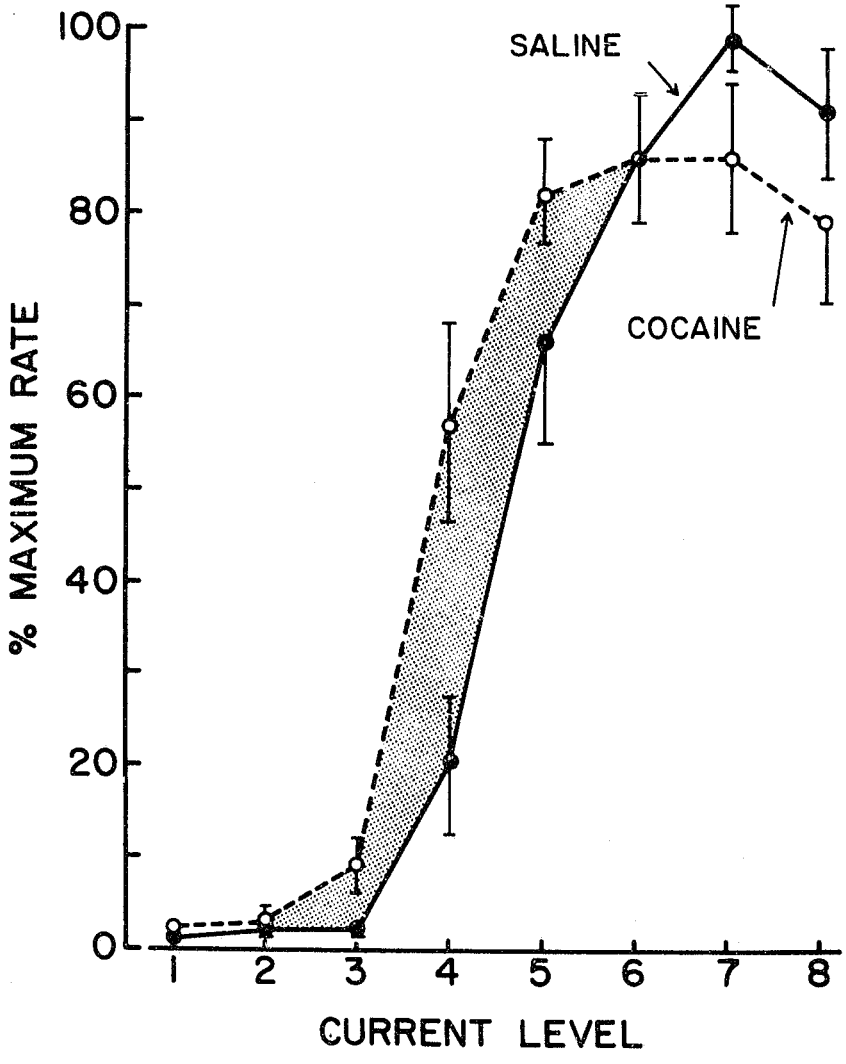

FIGURE 2. *Effects of intraperitoneally injected cocaine (10 mg/kg) in rats lever-pressing for brain stimulation reward from electrodes in the lateral hypothalamus*

NOTE: Cocaine was injected intraperitoneally 15 minutes prior to the test session. Rats were tested for 5 minutes at each of eight current levels in a descending series. Data are expressed as mean ± SEM of the percent of maximal responding for each rat under the saline condition (n=8).

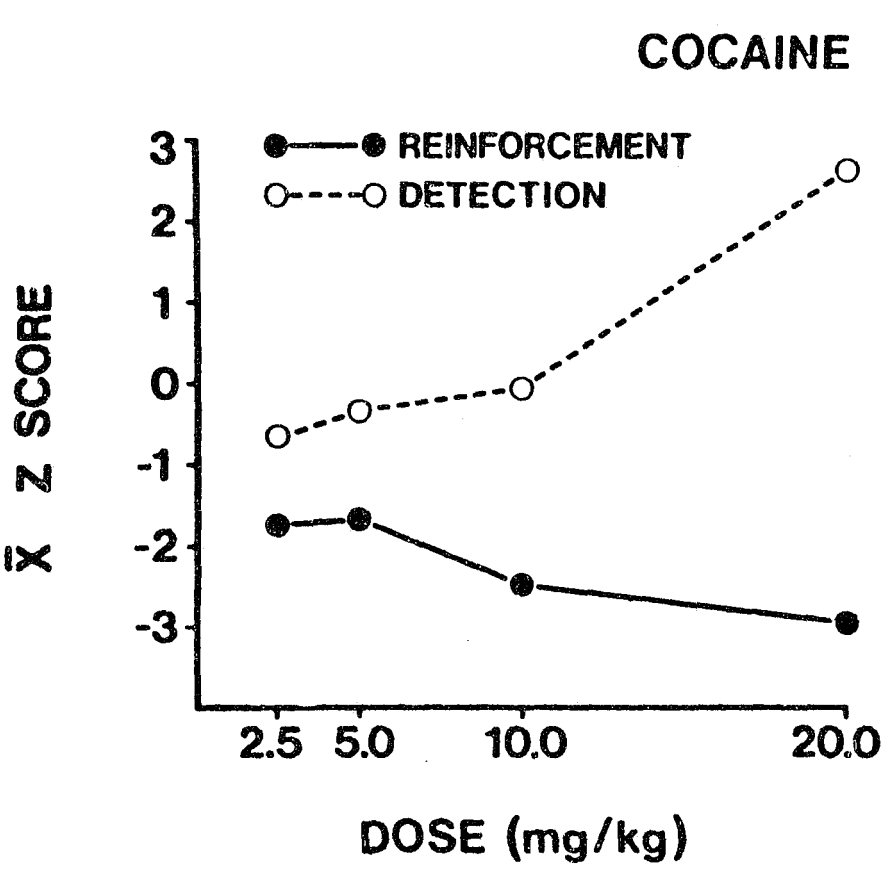

FIGURE 3. *Mean effect of various doses of cocaine on the threshold for brain stimulation reward and on the threshold for brain stimulation detection*

NOTE: Data are expressed as Z-scores based on the respective mean and standard deviation of the effects of saline (n=4).

SOURCE: Kornetsky and Bain 1982, Copyright 1982, the New York Academy of Sciences.

postsynaptic receptors. When the synthesis of catecholamines is blocked by administering alpha-methyl-para-tyrosine, an attenuation of the subjective effects of euphoria associated with psychomotor

stimulants occurs in man (Jonsson et al. 1971), and a blockade of the reinforcing effects of methamphetamine occurs in animals (Pickens et al. 1968). Furthermore, low doses of dopamine antagonists will increase response rates for intravenous injections of *d*-amphetamine (Risner and Jones 1976; Yokel and Wise 1975; Yokel and Wise 1976). Noradrenergic antagonists such as phenoxybenzamine, phentolamine, and propranolol have no effect on stimulant (amphetamine) self-administration (Risner and Jones 1976; Yokel and Wise 1976; DeWit and Wise 1977).

The partial blockade of dopamine receptors produced by low doses of dopamine antagonists has been hypothesized to reflect a partial blockade of the reinforcing effects of *d*-amphetamine (Yokel and Wise 1976; DeWit and Wise 1977). Thus, animals are thought to compensate for decreases in the magnitude of the reinforcer by increasing their self-administration behavior. Similar results have been observed on cocaine self-administration with alpha-flupenthixol (Ettenberg et al. 1982) (figure 4) and many other dopamine receptor antagonists including haloperidol, chlorpromazine, metoclopramide, thioridazine, and sulpiride (Roberts and Vickers 1984). Recently, the selective D-1 antagonist SCH 23390 has been shown to increase cocaine self-administration in rats at doses that did not impair motor function, whereas spiperone, a D-2-selective compound, produced only small increases in responding at doses close to those that reduced motor function (Koob et al. 1987a) (figure 5). These results suggest that dopamine receptor blockade, and particularly D-1 receptor blockade, may be involved in the reinforcing effects of psychomotor stimulants in rats. It should be noted, however, that the SCH 23390 compound failed to consistently produce this action when administered intravenously to rhesus monkeys self-administering cocaine (Woolverton 1986).

Lesion Studies

The role of dopamine in the reinforcing properties of psychomotor stimulants was extended by the observations that 6-OHDA lesions of the nucleus accumbens produced extinctionlike responding that extended into a long-lasting reduction in self-administration of cocaine and *d*-amphetamine over days (Roberts et al. 1977; Lyness et al. 1979). This effect was thought to be largely due to the depletion of dopamine, since rats pretreated with desmethylimipramine before the nucleus accumbens lesion (to protect norepinephrine and serotonin from destruction with the 6-OHDA) showed an identical decrease in cocaine self-administration (Roberts et al. 1980). Similar

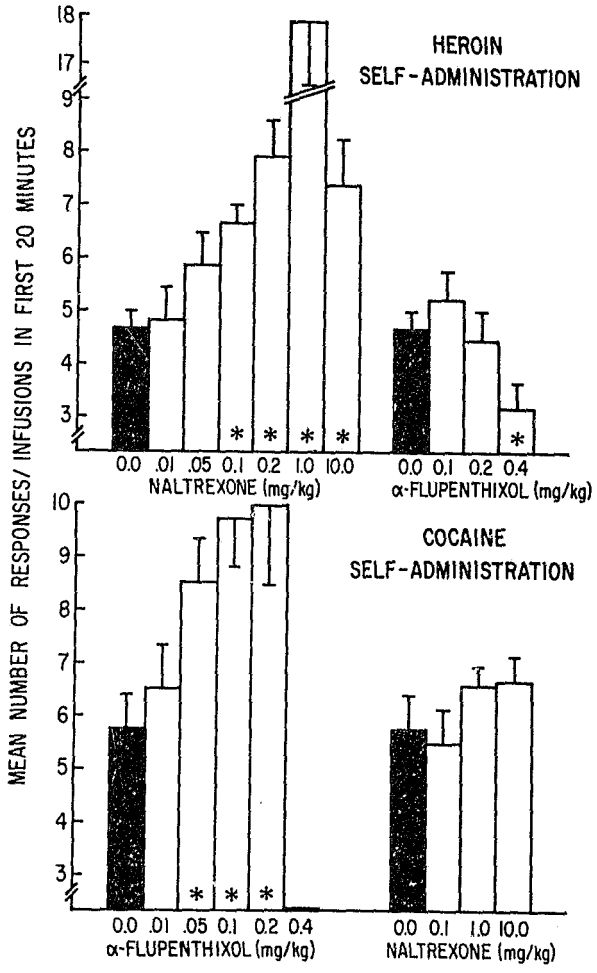

FIGURE 4. *The effects of naltrexone and alpha-flupenthixol on the loading dose (infusions in the first 20 minutes of the 3-hour test sessions) in both heroin- and cocaine-self-administering animals*

NOTE: Increases in responding were only observed in cocaine animals pretreated with opiate receptor antagonist. An asterisk in the base of a histogram indicates that the treatment dose was reliably different from the no-drug saline condition ($p<0.05$ Newman-Keuls test).

SOURCE: Ettenberg et al. 1982, Copyright 1982, Springer-Verlag.

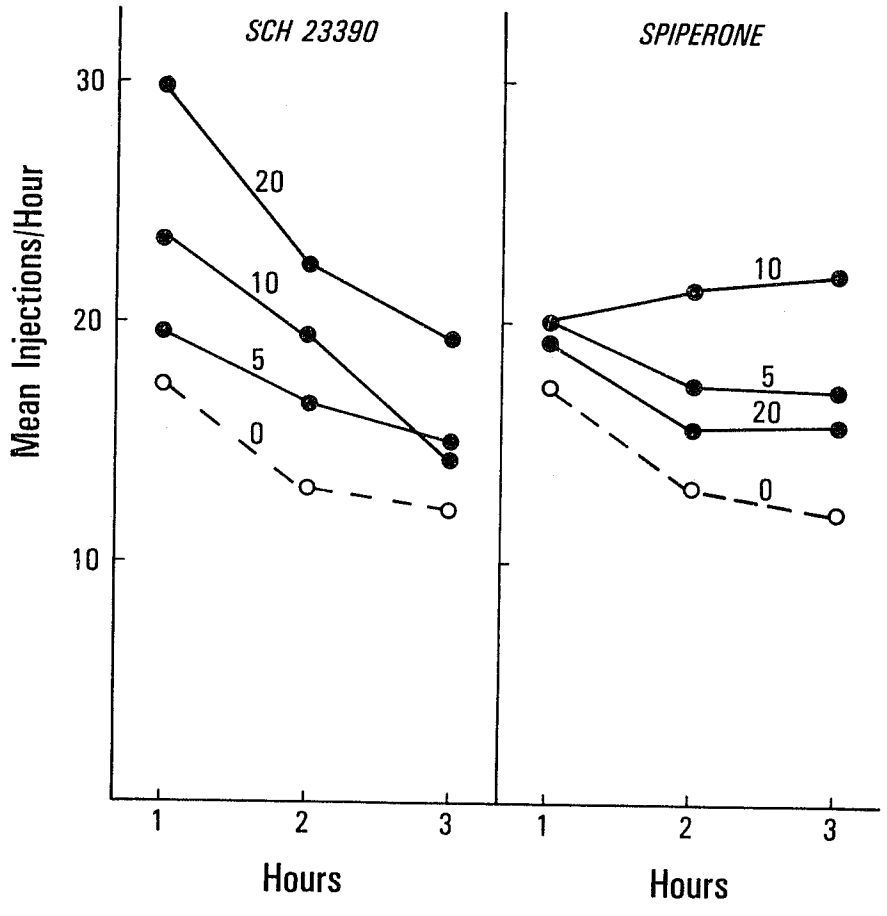

FIGURE 5. *Effects of the D-1 dopamine receptor antagonist SCH 23390 (left side) and the D-2 dopamine receptor antagonist spiperone (right side) on cocaine self-administration*

NOTE: Each point represents the average hourly intake of cocaine by injection (n=5). Doses are in micrograms per kilogram. For SCH 23390, doses of 5, 10, and 20 μg/kg significantly increased cocaine self-administration ($p<0.05$ Newman-Keuls test following ANOVA). For spiperone, the dose of 10 μg/kg significantly increased cocaine self-administration ($p<0.05$ paired t-test, overall ANOVA $p>0.05$).

SOURCE: Koob et al. 1987a, Copyright 1987, Elsevier Scientific Publishers Ireland Ltd.

results were obtained following 6-OHDA lesions of the ventral tegmental area (Roberts and Koob 1982). Subsequent studies have shown that 6-OHDA lesions of the frontal cortex (Martin-Iverson et al. 1986) and corpus striatum (Koob et al. 1987b) do not produce this decrease in responding for cocaine.

These results with intravenous psychomotor stimulant self-administration are consistent with earlier studies showing that the mesocorticolimbic dopamine system has an important role in mediating the activation associated with psychomotor stimulants (Kelly et al. 1975; Kelly and Iversen 1976; Roberts et al. 1975). For example, 6-OHDA lesions of the region of the nucleus accumbens block the locomotor activation due to amphetamine (Kelly et al. 1975; Roberts et al. 1975) and cocaine (Kelly and Iversen 1976). These lesions, however, do not block the locomotor activation associated with scopolamine and caffeine (Joyce and Koob 1981), heroin (Vaccarino et al. 1986), or corticotropin-releasing factor (Swerdlow and Koob 1985). This supports the hypothesis that these lesion effects on activation produced by psychomotor stimulants are not nonspecific changes in motor capability.

Nevertheless, the simple interpretation of a reduction in responding as a reward deficit is confounded in the self-administration studies by the observation that dopamine receptor antagonists produce an increase in responding, which has been interpreted as a decrease in the reinforcing properties of the drug. In the 6-OHDA lesion studies discussed above, partial lesions led instead to a partial reduction in responding. Further confounding the reinforcement deficit interpretation is the series of studies reported by Le Moal and colleagues showing that mesocorticolimbic dopamine lesions facilitate acquisition of *d*-amphetamine self-administration (Le Moal et al. 1979; Deminiere et al. 1984). Thus, other measures of reinforcing value are needed that do not depend on a simple continuous reinforcement procedure where each lever press produces a single intravenous injection.

In a recent study in our laboratory, rats that had been trained to self-administer cocaine intravenously were subjected to a progressive ratio procedure following 6-OHDA lesions to the nucleus accumbens or corpus striatum. The rats with lesions of the nucleus accumbens showed a significant decrease in the highest ratio for which they would respond to obtain cocaine (Koob et al. 1987b) (figure 6). Furthermore, rats with 6-OHDA nucleus accumbens lesions increased significantly the highest ratios for which they would self-administer

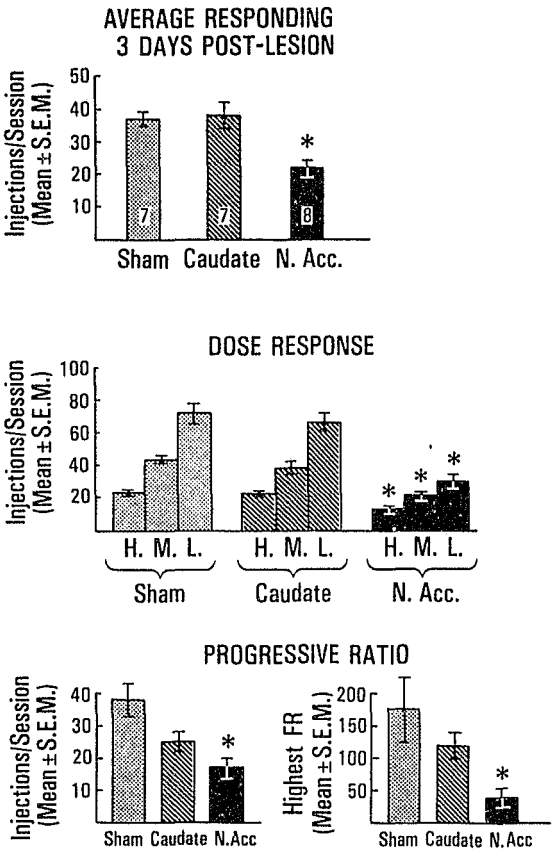

FIGURE 6. *Effects of 6-OHDA lesions to the nucleus accumbens and corpus striatum on responding in rats self-administering cocaine*

*Significantly different from sham group, $p<0.05$ Newman-Keuls test.

NOTE: Top panel shows continuous reinforcement data averaged for the first 3 days postlesion (mean ± SEM). Sham: vehicle (0.1 mg/ml ascorbic acid in saline-injected controls. Caudate: rats receiving 8 μg in 2μl of 6-OHDA injected into the nucleus accumbens. Middle panel shows the dose-effect functions for each group. H, two times the normal 0.75 mg/kg/injection dose; M refers to middle dose range, 0.75 mg/kg/ injection; L refers to one-half of the 0.75 mg/kg/injection dose. Bottom panel shows the mean injections and mean highest ratio obtained by each group on the progressive ratio schedule.

SOURCE: Koob et al. 1987b, Copyright 1987, Raven Press.

apomorphine using a similar progressive ratio procedure (Roberts and Vickers 1987), suggesting that the direct dopamine agonist had become more reinforcing in supersensitive 6-OHDA-lesion rats. This motivational probe thus avoids many of the problems associated with measuring simple rates of responding. The results in the progressive ratio test suggest that the decrease in rate of responding for cocaine observed previously does in fact represent a motivational deficit.

A few studies have examined the role of serotoninergic systems in mediating stimulant self-administration. In one study, animals were injected intracerebroventricularly with the serotonin neurotoxin 5,7-dihydroxytryptamine (80 to 90 percent forebrain serotonin depletion) prior to self-administration training. This lesion produced an increase in the amount of *d*-amphetamine self-administered during acquisition and after baseline stabilization. These rats had an increased tendency to overdose. The authors speculated that a generalized serotonin depletion produced "a decreased reinforcing effect of *d*-amphetamine" (Lyness et al. 1980, p. 940). This effect could not be replicated by 5,7-dihydroxytryptamine injections into the nucleus accumbens (Lyness et al. 1980). It is clear that general serotonin neurotoxin lesions change psychomotor stimulant self-administration in a manner opposite to that of dopamine neurotoxin lesions. However, without other behavioral tests, it is difficult to assess whether this reflects increases or decreases in the reinforcing effects of the psychomotor stimulant.

Recent work examining the functional output of the nucleus accumbens has established the region of the substantia innominata (ventral pallidum) as a critical connection in the expression of nucleus accumbens behavioral stimulation (Mogenson and Nielson 1983). For example, electrolytic or ibotenic acid lesions of the ventral pallidum block the supersensitive locomotor response to apomorphine produced in rats with destruction of dopamine terminals in the nucleus accumbens. In addition, this nucleus accumbens-ventral pallidum connection is thought to be GABAergic, since GABA infused into the ventral pallidum blocks the locomotor activation produced by direct application of dopamine into the nucleus accumbens (Mogenson and Nielson 1983), and low doses of the GABA agonist muscimol similarly block the supersensitive apomorphine response in nucleus accumbens 6-OHDA-lesion rats (Swerdlow and Koob 1984).

Studies designed to explore the role of the nucleus accumbens-ventral pallidum connection in the reinforcing effects of cocaine have recently been completed. Kainic acid lesions of the nucleus accumbens

produce decreases in rates of cocaine self-administration in rats trained on 1.5 mg/kg/injection cocaine (Zito et al. 1985). There was a high positive correlation between the degree to which responding decreased and the extent of the lesion in the nucleus accumbens (Zito et al. 1985). Similar effects have now been observed in rats with ibotenic acid lesions of the ventral pallidum (Hubner and Koob 1987). In this study, rats were trained to self-administer intravenous cocaine (0.75 mg/kg/injection) in 3-hour daily sessions on a fixed-ratio 5 schedule. After reaching steady baseline responding (±10 percent of the average for 3 consecutive days), rats received either bilateral injections of vehicle or ibotenic acid. Subjects receiving an ibotenic acid lesion in the ventral pallidum showed a significant decrease in cocaine self-administration when compared to vehicle-injected control animals. A cocaine dose-effect determination revealed an overall decrease in responding for cocaine for the lesion group, but the integrity of the typical dose-effect relationship was preserved. The effect of ibotenic acid lesions on cocaine self-administration was also assessed using a progressive ratio procedure. In this test, the lesion group displayed a significant decrease in the highest fixed-ratio value obtained. These results suggest that the neurons in the nucleus accumbens that mediate the reinforcing actions of psychomotor stimulants may project to the ventral pallidum, as do the neurons responsible for locomotor activation (Swerdlow and Koob 1985).

SUMMARY AND CONCLUSIONS

Neurochemical Substrates of Cocaine Reward

There is some significant overlap in the brain substrates that appear to be involved in cocaine's effect on brain stimulation reward and those sites involved in cocaine self-administration. Brain sites shown to be sensitive to the threshold-reducing effects of cocaine are in the medial forebrain bundle. The medial forebrain bundle represents one of the important substrates for brain stimulation reward, and lesions of the dopamine system ascending in the medial forebrain bundle disrupt intravenous cocaine self-administration. While work with brain stimulation reward has generally focused on the midbrain source of the medial forebrain bundle (the ventral tegmental area), self-administration studies have focused on the forebrain connections of the extrapyramidal motor system, the nucleus accumbens. Linking the two is the mesocorticolimbic dopamine system. Indeed, it may be this distinction that can explain the interesting effects of naloxone on psychomotor stimulant facilitation of brain stimulation. It will be

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of some interest to determine if the action of cocaine on brain stimulation reward and its interaction with opioids is site specific.

Implications for Cocaine Dependence

The brain dopamine system as a focus for cocaine reward leads to a number of important implications for our understanding of cocaine dependence and possible treatment. Hypothetically, this same system may be involved in the withdrawal symptoms of psychomotor retardation and depression following cocaine intake cessation. Also, this same system may be pathologically altered in drug-sensitive individuals (see studies of Le Moal et al. (1979) above). Drugs that act on the dopamine system may be useful in reducing the craving associated with the drug as well as withdrawal signs. Thus, the identification of an important, perhaps even critical, substrate for the rewarding effects of cocaine provides a focal point for more pre-clinical questions. This is not to argue that other neurotransmitters are not involved, but that these transmitters may ultimately act through this final common substrate.

Implications for Neural Substrates of Reward

What are the implications of this work with psychomotor stimulants for brain substrates of reward in general? There is little doubt that psychomotor stimulants are reinforcing in man, i.e., they are self-administered and they produce affective reactions variously described as euphoria or pleasure (Jonsson et al. 1971).

Since the neurochemical sites for psychomotor stimulant reward are likely to be the presynaptic dopamine terminals located in the region of the nucleus accumbens, frontal cortex, and other forebrain structures that originate from the ventral tegmental area, this system may be part of the circuitry involved in the processing of natural reinforcers. Note, however, that intracranial self-administration of cocaine is elicited from the frontal cortex, but not the nucleus accumbens (Goeders and Smith 1983). Thus, concomitant activation of structures other than the nucleus accumbens may be an important part of the circuitry involved in initiation of cocaine self-administration and reward.

In addition, these studies provide evidence to show that, in the rat, the neural/neurochemical substrates for processing the reinforcing and stimulant properties of psychomotor stimulants may be similar, if not identical. Parallel manipulations using dopamine receptor antagonists

and 6-OHDA lesions produce parallel results. How far this parallelism continues in further processing is under current investigation. However, this overlap brings additional impetus to earlier hypotheses relating reinforcement and motor function (Glickman and Schiff 1967). It may be that reinforcers or rewards that produce an activation of an organism rely on the same system that is exaggerated by psychomotor stimulants (Wise and Bozarth 1987). Whether all reward is processed through this same circuitry in a serial or parallel manner is an area of intense investigation and controversy (Wise 1982; Wise and Bozarth 1984; Koob 1987; Koob and Goeders 1988).

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Multiple Dopamine Receptors and the Behavioral Effects of Cocaine

W.L. Woolverton and M.S. Kleven

INTRODUCTION

Cocaine is a psychomotor stimulant and has behavioral effects that are typical of that class of drugs. In addition, it has been known for some time that cocaine blocks the uptake of monoamines into neurons. An assumption of behavioral pharmacology has been that this biochemical action in the brain is involved in most if not all of the behavioral effects of cocaine. In particular, a substantial amount of research suggests that a number of those effects are mediated by an increase in the concentration of the neurotransmitter dopamine (DA) in synapses in the brain.

To exert its effects, DA acts on DA receptors. In recent years, it has become clear that there is more than one type of receptor for DA. After some discussion as to whether there are two, three, or four DA receptors, the current consensus is that two are sufficient to account for most of the available data. If other receptor systems can serve as examples, it is likely that the ranks of DA receptors will swell again in the future. In the brain, these receptor subtypes have been designated, straightforwardly if somewhat unimaginatively, as D-1 and D-2. Originally they were characterized biochemically according to their relationship to the production of cAMP in brain homogenates (Kebabian and Calne 1979). D-1 receptors are positively linked to adenylate cyclase. Agonists at the D-1 receptor stimulate the production of cAMP. D-2 receptors were originally said not to be linked to adenylate cyclase but have recently been found to be negatively linked to adenylate cyclase, i.e., D-2 agonists can inhibit the production of cAMP (Stoof and Kebabian 1984). Defining the function of D-1 and D-2 receptors in the brain is currently a very active area of research.

The purpose of this paper is to briefly review the evidence that cocaine is an indirect DA agonist and to relate some of the behavioral effects of cocaine to its indirect action at D-1 or D-2 receptors. Our approach will be a pharmacological one: to compare the behavioral effects of cocaine to those of DA agonists with selective D-1 or D-2 or mixed actions and to examine the interaction of cocaine with antagonists with selective D-1 and D-2 or mixed actions. The basic premise is simple: if a D-1 agonist mimics a behavioral effect of cocaine and a D-1 antagonist blocks the effect, it suggests that D-1 receptors are involved in that effect.

It must be noted at the outset that there is precious little information available that bears directly on the role of DA receptor subtypes in the behavioral effects of cocaine. A major reason for this deficiency, surely, is that it has been less than a decade since we have been aware that there is more than one DA receptor. Although a selective D-1 agonist (SKF 38393) was discovered in 1976 (Setler et al. 1978), the fact that it was a selective D-1 agonist was not appreciated until later. Even now it is one of the very few D-1 agonists that cross the blood-brain barrier, a fact that makes problematic the determination of an *in vivo* potency series that is essential to the demonstration of a receptor-mediated effect. Moreover, an antagonist selective for the D-1 receptor (SCH 23390) has been available for only 5 years (Iorio et al. 1983). (Sadly, the days of drug names like arecoline, rauwolfia, and bufotenin seem to be gone.) It should also be said that the recent intense interest in cocaine has made the investigation of receptor mechanisms involved in its effects a viable research issue.

NEUROCHEMICAL EFFECTS OF COCAINE

Cocaine has been known for many years to block the uptake of monoamines (MacMillan 1959). Although initial research to demonstrate this effect was done in the periphery, it was not long before researchers postulated that this was the mechanism of action in the central nervous system (CNS). Indeed, cocaine has been shown to block the uptake of norepinephrine (NE), DA, and 5-hydroxytryptamine (5-HT) into presynaptic terminals *in vitro* (Koe 1976; Taylor and Ho 1978; Randrup and Braestrup 1977). Although it is often difficult to distinguish between release and blockade of uptake, it is generally believed that cocaine has little effect on monoamine release except at very high doses (Heikkila et al. 1975; Scheel-Kruger et al. 1977).

Of these neurochemical effects, attention has centered upon the DA reuptake-blocking properties of cocaine. DA levels in the caudate nucleus have been reported to increase by about 50 percent within 20 minutes after injection of 20 mg/kg cocaine (Pradhan et al. 1978; Bhattacharyya et al. 1979). Within 60 minutes after injection, these effects had returned to control values. Scheel-Kruger (1972) could not find an effect of moderate doses of cocaine (e.g., 25 mg/kg) on DA levels in rat brain 2 hours after injection. The reason for this relatively minor change in brain DA levels may be explained by a recent study. Church et al. (1987) used microdialysis to measure extracellular concentrations of DA in rat striatum. Cocaine produced as much as a ninefold increase in striatal DA, with a timecourse of approximately 1 hour. Other DA uptake inhibitors (nomifensine and benztropine) also increased extracellular DA with slight differences in maximal effect and duration of effect. Apparently, a rather large increase in extracellular DA was not detected in earlier studies using *ex vivo* analysis. This effect could be due to a decrease in DA metabolism subsequent to a blockade of DA reuptake. Cocaine has also been reported to increase DA turnover (Patrick and Barchas 1977), perhaps by stimulating tyrosine hydroxylase activity. More recently, cocaine has been found to increase the concentration of the DA metabolite HVA in striatum, an effect that also implies that turnover of DA was increased and would not be based solely upon uptake inhibition (Hanson et al. 1987).

Other neurochemical actions of cocaine may be involved in some of its behavioral effects. Although cocaine has been found to block the uptake of NE and 5-HT, concomitant with the increase in DA described above, there was a decrease in brain NE and 5-HT levels (Pradhan et al. 1978; Bhattacharyya et al. 1979). Within 20 minutes after injection, there was a 40 percent depletion of 5-HT in the diencephalon-midbrain and pons-medulla and a 20 percent decrease of NE levels in both regions. Within 60 minutes after injection, these effects had returned to control values. The decrease in NE and 5-HT, an effect opposite that seen with DA, may reflect differences in neuronal compensation and/or effects of cocaine beyond simple blockade of monoamine reuptake. Recent studies of cocaine binding by Reith and colleagues (Reith et al. 1983; Reith et al. 1986) suggest the existence of several separate cocaine binding sites, each associated with uptake of either DA or 5-HT. The existence of 5-HT-related binding sites may explain the effects of cocaine on 5-HT uptake *in vitro* (Koe 1976; Taylor and Ho 1978; Randrup and Braestrup 1977). It should be noted, however, that antidepressant drugs that block neuronal uptake of NE and/or 5-HT, but do not affect DA

uptake, are not psychomotor stimulants. Moreover, cocaine has a unique pharmacology, since it is a local anesthetic and decreases 5-HT synthesis (Knapp and Mandell 1972). It should be emphasized that cocaine's behavioral effects may involve any or all of these neuronal actions.

BEHAVIORAL EFFECTS OF COCAINE

It has long been known that cocaine is a psychomotor stimulant. Research with humans in the late 19th century demonstrated this fact unequivocally (Freud 1884). Some 30 years later, when animal researchers began working with cocaine, it was noted that cocaine stimulated locomotor activity and produced stereotyped behavior in rats and monkeys (Downs and Eddy 1932; Tatum and Seevers 1929). More recently, it has been demonstrated that cocaine is self-administered by a wide variety of species, and methods have been developed for studying its prominent subjective effects, including euphoria. These effects are certainly critical determinants of the apparently exceptional abuse potential of cocaine.

Many of the behavioral effects of cocaine are believed to be the result of enhanced DA neurotransmission in the CNS (Wise 1984). A variety of types of experimental evidence converge upon this point. Two of the major factors are that DA agonists have many of the same behavioral effects as cocaine and that DA antagonists can block these effects of DA agonists and of cocaine. What follows is a review of the data showing that DA receptors are involved in several of the more prominent behavioral effects of cocaine. To the extent possible, we will relate these to the DA receptor subtypes that may be involved. In several instances in which no data are available for cocaine we will consider what, if anything, is known about *d*-amphetamine, a compound that is often behaviorally interchangeable with cocaine. In addition, we will consider the data examining other neurochemical mechanisms, particularly noradrenergic, where they have been implicated.

DA RECEPTORS AND THE REINFORCING EFFECTS OF COCAINE

If a response that resulted in drug delivery increases in frequency, the drug is defined as a positive reinforcer. Reinforcing effects are a primary determinant of dependence potential. It has become clear that cocaine is a particularly strong positive reinforcer. As with the other behavioral effects of cocaine, it is suspected that this effect is due to its effects on DA neurotransmission in the brain.

If DA receptors mediate the reinforcing effects of cocaine, then agonists that directly stimulate these receptors would be expected to function as positive reinforcers. Baxter et al. (1974) reported that the DA agonist apomorphine functioned as a positive reinforcer in rats. Rats prepared with intravenous catheters self-administered apomorphine at rates substantially above those maintained by the drug vehicle. Apomorphine injections within a session were regularly spaced, a pattern that was reminiscent of the pattern of cocaine self-administration. Moreover, DA antagonists increased the rate of apomorphine self-administration just as has been found with cocaine self-administration (see below), further supporting the notion that DA receptors are involved in this reinforcing effect (Baxter et al. 1974; Gill et al. 1978; Yokel and Wise 1978). This was the first evidence that a drug that is a direct agonist at DA receptors could function as a positive reinforcer. Since that time, the finding has been confirmed several times in rats (Wise et al. 1976; Yokel and Wise 1978).

The discovery of a selective D-1 agonist (SKF 38393) and antagonist (SCH 23390) allowed us to begin to examine the role of DA receptor subtypes in the reinforcing effects of cocaine. In one study (Woolverton et al. 1984), rhesus monkeys were prepared with intravenous catheters and allowed to self-administer cocaine or *d*-amphetamine in daily experimental sessions that were 2 or 3 hours long. When self-administration was stable under these baseline conditions, drug vehicle or a dose of a DA agonist was made available for several consecutive sessions. When drug vehicle was available, responding declined to low levels over a period of 4 to 10 sessions, as expected. When a DA agonist was available, responding was maintained at levels above those maintained by the vehicle in most of the monkeys. The single, clear exception was the D-1 agonist SKF 38393. In all cases, responding for this agonist occurred at or below vehicle levels over a wide range of doses. The other drugs had in common agonist actions at the D-2 receptor. In fact, the compound that was most reliably self-administered, piribedil, is the most selective in terms of D-2 actions. This study clearly implicated D-2 receptors in the reinforcing effects of cocaine.

Experiments with antagonists also suggest that DA receptors are involved in the reinforcing effects of cocaine. The first experiments to test this hypothesis involved administering catecholamine receptor blockers before a session of cocaine self-administration. Wilson and Schuster (1972) showed that when chlorpromazine (CPZ) was given before a session of cocaine self-administration, the rate of cocaine self-administration increased as though the unit dose of cocaine had

been reduced. That is, it appeared that the effects of cocaine had been at least partially antagonized by CPZ. Of course, since CPZ blocks a multitude of receptors, it was difficult to relate this result to its effect at any single type of receptor. Nevertheless, the results were consistent with the hypothesis that catecholamine receptors are involved in the reinforcing effects of cocaine.

Research since this initial experiment has tended to implicate DA receptors in this effect. Haloperidol, perphenazine, pimozide, flupenthixol, (+)-butaclamol, sulpiride, thioridazine, and metoclopramide, all primarily DA receptor blockers, have been found to increase the rate of cocaine self-administration in several different species (de la Garza and Johanson 1982; Gill et al. 1978; Johanson et al. 1976; de Wit and Wise 1977; Risner and Jones 1976; Roberts and Vickers 1984; Woods et al. 1978). An interesting effect that has not been systematically pursued was the discovery by Wilson and Schuster (1973) that atropine produces a similar increase in cocaine self-administration. On the other hand, DA agonists have been shown to have the opposite effect. The direct agonist bromocriptine (Hubner and Koob 1987; Woolverton and Kleven 1987), as well as the indirect DA agonists amphetamine and phenmetrazine reduced the rate of cocaine self-administration when they were administered in the same manner as the DA antagonists described previously (Wilson and Schuster 1973; Herling et al. 1979). In addition, a variety of other compounds including clozapine (Roberts and Vickers 1982), pargyline (Wilson and Schuster 1974), physostigmine (de la Garza and Johanson 1982; Wilson and Schuster 1973), morphine, pentobarbital, and imipramine (Wilson and Schuster 1973; Herling et al. 1979) have been found to have no effect on or to reduce cocaine self-administration.

Virtually all of the compounds that have been shown to increase cocaine self-administration are primarily D-2 antagonists. The availability of a selective D-1 antagonist (Iorio et al. 1983) made it possible to compare the effects of this compound on cocaine self-administration with those of D-2 antagonists. In another study (Woolverton 1986), rhesus monkeys were allowed to self-administer cocaine during daily 2-hour experimental sessions. When behavior was stable, they were injected with the D-1 antagonist SCH 23390 or the D-2 antagonist pimozide at a timepoint before the session that assured maximal effect during the session. Intermediate doses of pimozide generally increased the rate of responding for cocaine in the same manner as has been found with other DA antagonists. On the other hand, SCH 23390 usually failed to increase the rate of responding for cocaine. A high dose of pimozide resulted in a pattern of

responding for cocaine that was similar to what was seen when saline was available for self-administration (figure 1). In contrast, a dose of SCH 23390 that had comparable rate-reducing effects completely suppressed responding early in the session and did not affect cocaine self-administration later in the session. There was no suggestion of pattern of responding similar to that seen when saline was available.

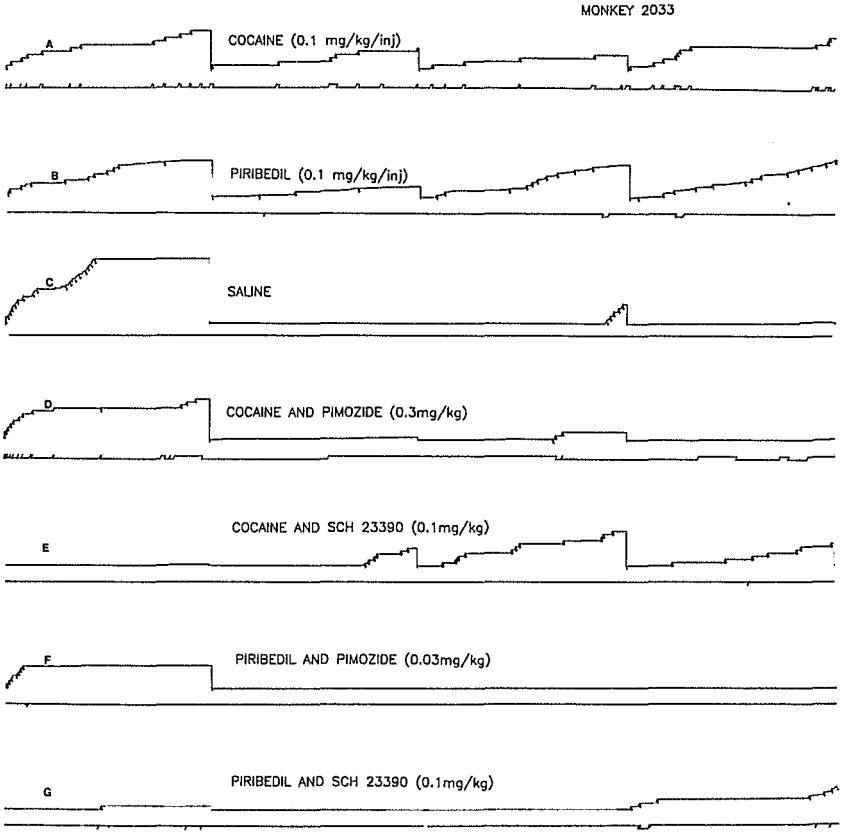

FIGURE 1. *Effects of a high dose of pimozide or SCH 23390 on responding maintained by cocaine or piribedil in rhesus monkeys*

NOTE: Diagonal marks represent drug injections. Responding on the inactive lever was marked by the lower pen. DA antagonists were given IV either 2 hours (pimozide) or 30 minutes (SCH 23390) before the 2-hour session.

The antagonists had effects on responding for the D-2 agonist piribedil that were similar to their effects on cocaine-maintained responding. These results are consistent with our findings with agonist self-administration in suggesting a D-2 receptor role in the reinforcing effects of cocaine. Goeders et al. (1986) reached similar conclusions using rats as experimental subjects. The D-2 antagonist sulpiride eliminated cocaine-maintained responding whereas SCH 23390 did not.

Although these experiments with antagonists are consistent with the hypothesis that D-2 receptors are involved in the reinforcing effects of cocaine, they must be considered equivocal evidence of antagonism of the reinforcing effects of cocaine. Rate of drug self-administration under the conditions utilized in these experiments is determined by a number of drug effects in addition to the reinforcing effects (Johanson 1978). For instance, if the effects of cocaine that tend to reduce rate of responding regardless of the reinforcing stimulus were antagonized, rate of responding for cocaine would increase as well. In fact, Herling and Woods (1980) concluded that the evidence from behavioral studies was most consistent with a mutual antagonism of rate-reducing effects of cocaine and neuroleptics. In addition, we have recently examined the effects of SCH 23390 or pimozide on behavior maintained under a multiple schedule of food or cocaine delivery (Woolverton and Virus, submitted). Although it was possible to reduce cocaine self-administration with either of these compounds as though the reinforcing effects of cocaine were blocked, reductions occurred only at doses that comparably reduced responding maintained by food. Thus, the effects of DA antagonist were not specific for cocaine but were generalized rate-reducing effects.

As to NE mechanisms in the reinforcing effects of cocaine, both direct and indirect NE agonists have been found not to be self-administered by animals (Risner and Jones 1976; Woolverton 1987). In addition, antagonists at NE receptors, including phenoxybenzamine, phentolamine, propranolol, and prazosin, have been shown to have no effect on or to reduce cocaine self-administration (de Wit and Wise 1977; Goldberg and Gonzalez 1976; Wilson and Schuster 1974; Woolverton 1987). These findings suggest that the NE actions of cocaine do not play a prominent role in its reinforcing effects.

In summary, much of the available evidence suggests that a D-2 receptor action is involved in the reinforcing effects of cocaine. It should be pointed out that Koob et al. (1987) recently reported that

SCH 23390 consistently increased the rate of cocaine self-administration in rats, while the D-2 antagonist spiperone only occasionally had this effect. These results are in direct contrast to our findings with rhesus monkeys. Although the reasons for this discrepancy are unclear, it is possible that species differences played a role. It is possible as well that the short duration of action of SCH 23390 played a role in the negative effects we observed in rhesus monkeys. In view of these conflicting results and the data concerning the effects of SCH 23390 on the discriminative stimulus properties of cocaine to be described in the next section, the effects of D-1 antagonists on the reinforcing effects of cocaine bear further investigation. We are currently investigating the effects of continuous infusion of SCH 23390 on cocaine self-administration.

DA RECEPTORS AND THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE

Discriminative stimuli are stimuli that set the occasion for a particular behavior to be reinforced. For instance, if a behavior is reinforced in the presence of a red light, the red light is called the discriminative stimulus for that response. It has been shown repeatedly in a number of species that a variety of psychoactive drugs, including cocaine, can function as discriminative stimuli. If one behavior is reinforced following an injection of cocaine and a second behavior reinforced following an injection of saline, the drug comes to function as the discriminative stimulus that controls differential responding. This paradigm has proven quite useful for classifying drugs according to similarities in their discriminative stimulus properties. Drugs that substitute for or block the effects of the training drug may be considered to provide information relevant to the mechanism of action of the training drug. In addition, since the classification of drugs by animals trained in this paradigm is similar to the classification of drugs according to their subjective effects in humans, the paradigm is considered by many to be useful as an animal model of subjective effects (Schuster et al. 1981). When considered together, these two types of information may provide an indication of the central actions involved in the subjective effects of a drug. To the extent that subjective effects play a role in dependence potential, results of these experiments may help us understand the central mechanisms involved in cocaine dependence.

Initial investigations of the central mechanisms of the discriminative stimulus properties of cocaine examined the role of DA and DA receptors by testing apomorphine in cocaine-trained animals.

Colpaert et al. (1976) and McKenna and Ho (1980) have reported that the D-2 agonist apomorphine substituted for 10 mg/kg cocaine in rats, findings that were consistent with the hypothesis that DA and DA receptors play a primary role in the discriminative stimulus properties of cocaine. However, further research has not supported these findings. In a later study, Colpaert et al. (1979) tested the D-2 agonists apomorphine, piribedil, and bromocriptine in rats trained to discriminate 10 mg/kg cocaine from saline. All three drugs only partially substituted for cocaine. The most complete substitution for cocaine was seen with apomorphine, which resulted in a maximum of 75 percent cocaine-appropriate responding. Piribedil engendered a maximum of 57 percent, and only 29 percent drug-lever responding was seen with bromocriptine. Similarly, Jarbe (1981) reported a maximum of 21 percent cocaine-appropriate responding with apomorphine in pigeons trained to discriminate 5.6 mg/kg cocaine from saline. In a later paper, Jarbe (1984) reported that apomorphine induced 58 percent drug-key responses in pigeons trained to discriminate 3.0 mg/kg cocaine from saline. Similar results in pigeons trained to discriminate 2.0 mg/kg cocaine from saline were found by de la Garza and Johanson (1985). Thus, virtually all of the available data demonstrate, at best, a partial substitution of D-2 agonists for cocaine.

Although there is substantially less data available with D-1 agonists, the story to this point is similar to that with D-2 agonists. Wood and Emmett-Oglesby (1987) have found that the D-1 agonist SKF 38393 partially substitutes for cocaine in rats trained to discriminate 10 mg/kg cocaine from saline. The results are consistent with what has been found with this agonist in animals trained to discriminate *d*-amphetamine from saline. We have found that SKF 38393 partially substitutes for 0.5 mg/kg *d*-amphetamine in rats (figure 2) and monkeys (Woolverton 1984).

Unfortunately, findings of partial substitution make it necessary to equivocate on conclusions. The data would support the conclusion that a D-1 or a D-2 agonist partially reproduces the effects of cocaine. It should be noted that this partial substitution was often the result of averaging different levels of responding in different animals rather than the result of a consistent intermediate level of responding in all animals. It is possible, therefore, that different animals learned to discriminate different components of what is a complex stimulus, which includes both D1 and D-2 actions. Both possibilities are consistent with the conclusion that D-1 and D-2 receptors are involved in the discriminative stimulus properties of

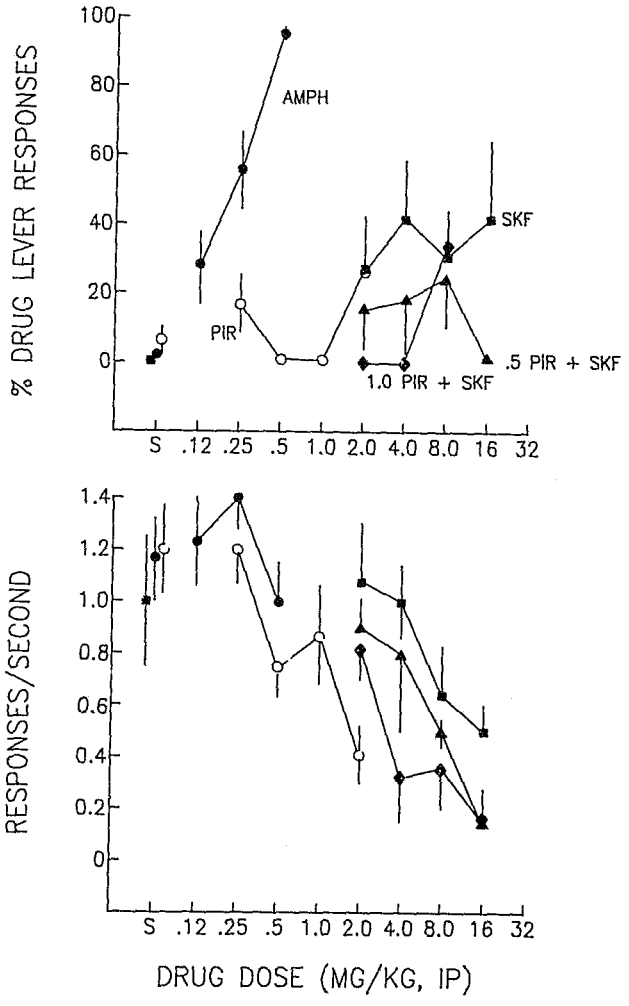

FIGURE 2. *Effects of SKF 38393 and piribedil alone or in combination in rats trained to discriminate 0.5 mg/kg d-amphetamine from saline*

NOTE: Drugs were injected IP 10 minutes before the 15-minute session. When combinations were given they were administered in separate syringes, one immediately after the other.

cocaine and raise the possibility that a combination of D-1 and D-2 agonists might substitute for cocaine. Although this has not been

tested with cocaine, we have done this experiment with *d*-amphetamine, a drug that consistently substitutes for cocaine as a discriminative stimulus. When the D-2 agonist piribedil was combined with the D-1 agonist SKF 38393 in rats trained to discriminate *d*-amphetamine from saline, partial substitution was again the result even up to doses that reduced rate of lever pressing (figure 2). Thus, a combination of D-1 and D-2 actions was not similar to *d*-amphetamine. Alternatively, it must be considered that partial substitution may often represent a nonspecific effect of a drug that is not like the training drug and at the same time is not like saline. If this interpretation is accurate, it suggests that DA receptors are not involved in this effect of cocaine. It should also be mentioned at this point that the neuropharmacology of a drug surely changes with dose. A most graphic behavioral demonstration of this effect was provided by Stolerman and D'Mello (1981), who demonstrated that apomorphine substituted for a high dose of *d*-amphetamine but not for a low dose and only partially for an intermediate dose. Such an effect is possible with cocaine. Thus, although DA receptor mechanisms are not clear-cut when 10 mg/kg cocaine is used as the training dose, they may be more apparent at higher doses. Clearly, further research is needed to thoroughly address these issues.

Experiments with antagonists have also been used to examine receptor mechanisms in the discriminative stimulus properties of cocaine. As with other pharmacological effects, a competitive antagonist of cocaine would be expected to cause a parallel shift of the cocaine dose-response function to the right. Unfortunately, in most of these experiments, only the effects of combining an antagonist with the training dose of cocaine were considered. Colpaert et al. (1976) reported that haloperidol failed to block the effects of 5.0 mg/kg cocaine. In contrast, Järbe (1978) reported that pimozide, haloperidol, and CPZ all reduced the percentage of drug responses in rats trained to discriminate 4.0 mg/kg cocaine from saline in a T-maze. Colpaert and colleagues showed that haloperidol, pimozide, and spiperone could reduce the percentage of responses that occurred on the drug lever following the training dose of cocaine (10 mg/kg) in rats (Colpaert et al. 1978a; Colpaert et al. 1978b). The most commonly reported effect has been a reduction in drug-appropriate responding with the training dose of cocaine from 100 percent to approximately 50 percent; that reduction occurred only at doses that substantially reduced ongoing behavior. Complete saline-appropriate responding has not been reported with combinations of a D-2 antagonist and cocaine. In the single case where this was examined, the dose-response function for cocaine was shifted to the right, and the

maximum effect was reduced from 100 percent to approximately 50 percent when cocaine was combined with pimozide or haloperidol (McKenna and Ho 1980). These results are suggestive of a noncompetitive antagonism of the discriminative stimulus properties of cocaine by D-2 antagonists. Unfortunately, it is necessary to equivocate over these findings of partial antagonism in much the same way as over partial substitution with DA agonists.

The effects of the D-1 antagonist SCH 23390 on the discriminative stimulus properties of cocaine are being examined in experiments currently in progress in our laboratory (Kleven et al., in press). Rhesus monkeys were trained to discriminate cocaine from saline and were injected with combinations of SCH 23390 and cocaine. The dose-response function for cocaine was shifted fourfold to eightfold to the right when combined with SCH 23390. Two important factors should be noted with the D-1 antagonist that have not been apparent with D-2 antagonists. The first is that drug-lever responding was consistently reduced from 100 percent to 0, evidence for complete blockade of the effects of cocaine. Further, the D-1 antagonist appeared to function as a competitive antagonist. That is, the dose-response function for cocaine was shifted parallel to the right. Similar results have been found with *d*-amphetamine as a discriminative stimulus in rhesus monkeys (Woolverton et al. 1987) and rats (Nielsen and Jepsen 1985). These data strongly suggest that D-1 receptors are somehow involved in the discriminative stimulus properties of cocaine and, perhaps, of psychomotor stimulants in general. Further, the data suggest that this novel group of compounds (i.e., D-1 antagonists) may function as antagonists of the subjective effects of cocaine. We feel that these are very exciting findings.

In summary, research with DA agonists and antagonists has demonstrated partial substitution for cocaine by D-2 agonists and, at best, partial blockade of the effect with D-2 antagonists. Although these data are consistent with a role for D-2 receptors in the discriminative stimulus effects of cocaine, the data could not be described as conclusive. Substitution data with a D-1 agonist are similar to those with D-2 agonists, and the limited amount of data with D-1 antagonists suggests a role for D-1 receptors as well.

The possibility that other mechanisms are involved in the discriminative stimulus properties of cocaine should also be considered. Early research with cocaine suggested that NE was probably not important. Beta adrenergic agonists either failed to substitute or only partially

substituted for cocaine in rats (Colpaert et al. 1979) and the NE antagonists phenoxybenzamine (alpha), propranolol (beta), and sotalol (beta), have been reported not to block the discriminative stimulus properties of cocaine (McKenna and Ho 1980; Jarbe 1978). However, more recent research with *d*-amphetamine has made it clear that it is important to consider the role of NE in the discriminative stimulus properties of cocaine. The indirect NE agonist nisoxetine has been shown to substitute for *d*-amphetamine as a discriminative stimulus in several species (Evans and Johanson 1987; Snoddy and Tessel 1983; Woolverton 1984) and the alpha NE antagonist prazosin has been reported to antagonize the discriminative stimulus properties of *d*-amphetamine in mice (Snoddy and Tessel 1985). Moreover, Colpaert et al. (1980) reported that MAO-B inhibitors substitute for cocaine and postulated that endogenous beta-phenylethylamine mediates the effects of cocaine, a finding that should be pursued. Finally, it should be mentioned that the antagonists cinanserin (5-HT) and atropine (ACH) did not block the discriminative stimulus properties of cocaine (McKenna and Ho 1980). Research with these neurotransmitter systems is extremely limited and should be expanded (Lakoski and Cunningham, this volume).

DA RECEPTORS AND OTHER BEHAVIORAL EFFECTS OF COCAINE

Among the other behavioral effects of cocaine are stimulation of locomotor activity, generation of stereotyped behavior, and reduction in food intake (Scheel-Kruger 1972; van Rossum and Simons 1969; Woolverton et al. 1978). In addition, cocaine disrupts schedule-controlled behavior (Herling et al. 1979). There is considerable evidence that CNS DA is involved in these effects (Creese and Iverson 1975; Kelly and Iverson 1976; Menon et al. 1967; Ziegler et al. 1972). Like cocaine, the DA agonists apomorphine, piribedil, and LY141865 have been shown to stimulate locomotor activity (Dourish 1983; Fuller et al. 1983; Offermeier and van Rooyen 1986). All are primarily D-2 agonists. On the other hand, the D-1 DA agonist SKF 38393 has little or no effect on locomotor activity in normal rats (Breese et al. 1985). A number of different investigators have reported that DA antagonists can block cocaine-induced locomotor activity. The D-2 antagonists pimozide and haloperidol completely antagonized the hyperactivity induced by cocaine and its analogs in rats and mice (Bhattacharyya et al. 1979; Heikkila et al. 1979; Scheel-Kruger et al. 1977). Although these results suggest that D-2 receptors are involved in the locomotor effects of cocaine, interpretation of the interactions between cocaine and D-2 antagonists is somewhat problematic. D-2 antagonists themselves reduce locomotor

activity, an effect that may give the appearance that locomotor stimulation by cocaine is blocked.

At higher doses, psychomotor stimulants, including cocaine, induce a syndrome of repetitive movements termed stereotyped behavior. Although in some sense this could be considered locomotor activity, it is generally treated as a different category of behavioral effect. Available data suggest different areas of the brain are involved in locomotor effects and stereotyped behavior (Kelly and Iversen 1976). High doses of D-2 agonists induce stereotyped behavior, and D-2 antagonists block the stereotyped behavior produced by cocaine and by D-2 agonists (Porsolt et al. 1982; Scheel-Kruger et al. 1977). Although D-1 agonists have been reported to induce a syndrome of repetitive grooming and mouth movements, these effects are apparently not stereotypic in nature (Molloy and Waddington 1987; Rosengarten et al. 1983; Starr and Starr 1986).

These studies are consistent in suggesting that a D-2 receptor action is involved in the locomotor stimulant effects of cocaine. However, it would be premature to suggest that the D-1 receptors do not play a significant role. Although the effects of the D-1 antagonist SCH 23390 on cocaine-induced locomotor stimulation or stereotypy have not been determined, Mailman et al. (1984) reported that SCH 23390 blocked *d*-amphetamine-induced locomotor effects. In addition, the behavioral effects of D-2 agonists can be blocked by the D-1 antagonist SCH 23390 (Mailman et al. 1984; Pugh et al. 1985; Walters et al. 1987). For instance, it has been reported that D-1 and D-2 agonists must be combined to produce the full spectrum of DA-mediated behaviors, including stereotyped behaviors (Arnt et al. 1987; Braun and Chase 1986). It is also of interest to note that the D-1 agonist stimulates locomotor activity in rats with lesions of the nigrostriatal pathway (Breese et al. 1985; Setler et al. 1978), suggesting that D-1 receptors do have some role in the regulation of DA-mediated effects. A number of investigators have interpreted these data to suggest that there are important interactions between D-1 and D-2 receptors in the expression of DA-mediated behavioral effects and that D-1 receptors are essential to the expression of those effects. Thus, a growing body of evidence suggests that D-1 receptors may be involved in the expression of dopaminergic drug effects. The role of D-1 receptors in the locomotor effects of cocaine is an area where research is needed.

Regarding other behavioral effects of cocaine, both D-1 and D-2 agonists can reduce food intake in the same manner as cocaine

(Gilbert and Cooper 1985; Barzaghi et al. 1973). Since this is often a nonspecific effect, it probably reveals little about receptor mechanisms. The interaction of cocaine with DA antagonists has not been examined in a food intake paradigm. It has been shown, however, that a D-1 antagonist can block the effects of amphetamines on food intake (Gilbert and Cooper 1985). The data with D-2 antagonists is more controversial, perhaps because there is more of it (Foltin et al. 1983; Gilbert and Cooper 1985). Similarly, apomorphine has some effects of schedule-controlled behavior that are similar to those of cocaine (Abelson and Woods 1980), although the specificity of these effects must be questioned as well. The effects of D-1 agonists on schedule-controlled responding have not, to our knowledge, been reported. The effects of cocaine on schedule-controlled behavior that can be partially blocked by D-2 antagonists and a mutual antagonism of rate-reducing effects of cocaine and D-2 antagonists has been postulated to account for these effects (Herling and Woods 1980). Similarly, in the Kleven et al. (in press) drug discrimination experiment, cocaine clearly reversed the effects of SCH 23390 on response rate and vice versa. In addition, NE may be important in the effects of cocaine on schedule-controlled behavior. In a recent paper, Tessel and Barrett (1986) showed that prazosin could antagonize the effects of cocaine on operant behavior. Further research is needed to follow up these provocative findings.

SUMMARY AND CONCLUSIONS

Cocaine has a number of behavioral effects that are typical of psychomotor stimulants and likely involve DA mechanisms in the brain. These effects include, but are not limited to, reinforcing effects, discriminative stimulus effects, locomotor effects, effects on food intake, and effects on schedule-controlled behavior. The available data suggest that D-2 receptors are involved in the reinforcing effects of cocaine, although the jury is still out concerning the role of D-1 receptors. NE receptors apparently do not play a major role in this effect. The role of D-2 receptors in the discriminative stimulus effects is unclear because of partial substitution and partial blockade effects with D-2 agonists and antagonists, respectively. Data with D-1 agonists and antagonists suggest that D-1 receptors may play a necessary-but-not-sufficient role in the discriminative stimulus effects of cocaine. Moreover, results in several species suggest that NE may be important in this effect of cocaine. Very little data currently exist concerning the role of DA receptor subtypes in the other behavioral effects of cocaine. However, data with *d*-amphetamine suggest that D-1 as well as D-2 receptors should

be investigated. In addition, there is evidence to suggest that NE is involved in some of cocaine's effects as well, an action that should be considered further.

So, what mediates the behavioral effects of cocaine? Clearly, the answer is not a simple one, basically because the CNS pharmacology of cocaine is complex. In addition to the effects we have concentrated on, cocaine has effects on other neurotransmitters and local anesthetic effects that must be considered. It is impossible to say that any one pharmacological effect of cocaine mediates its behavioral effects, indeed even that it mediates any one behavioral effect. In fact, the word "mediates" is surely virtually meaningless in the context of the CNS. Perhaps the best that can be said about the role of DA receptors in the reinforcing effects of cocaine is they are likely a link in a chain of events that ultimately results in the sensations produced by cocaine. Surely numerous other, perhaps more critical, links remain to be discovered. It is an exciting time to be doing research with cocaine.

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Neurobehavioral Pharmacology of Cocaine

Steven I. Dworkin and James E. Smith

INTRODUCTION

Behavioral pharmacologists have developed five behavioral screens that are widely used to investigate the abuse liability of pharmacological agents. These procedures include drug self-administration, conditioned place preference, drug discrimination, electrical brain stimulation, and the use of schedule-controlled behavior. Each of the five behavioral procedures has its own strengths and weaknesses, a fact that necessitates the use of several procedures to thoroughly investigate the behavioral effects of any drug. All of these procedures have been, or are currently, used to investigate the abuse liability of cocaine and the neurobiological mechanisms involved in drug abuse. This chapter will review procedures used to investigate the reinforcing, rate modulating, and stimulus effects of cocaine. Furthermore, this chapter provides an example of an integrated neurobiological approach to determining the neurobiology of cocaine abuse.

DRUG SELF-ADMINISTRATION

The drug self-administration procedure that was developed more than 30 years ago has been used extensively to determine the reinforcing effects of drugs. Several different routes of drug administration, including oral, intramuscular, intranasal, intravenous, inhalation, and direct delivery into various brain sites have been studied using several species of subjects including humans (Brady and Lukas 1984). A typical experimental protocol involves surgically preparing subjects with chronic indwelling catheters that allow for the intravenous presentation of a drug. The subject is then placed in an operant conditioning chamber containing a response lever. Responses on this

lever result in the activation of a syringe pump that injects a precise amount of the drug into the subject.

Studies investigating drugs that are self-administered by nonhuman subjects have provided a list of pharmacological agents that are abused by humans. This procedure thus provides a general screen for substances that have a potential for being abused. Cocaine in particular has been shown to quickly engender robust self-administration patterns and has become the standard to compare other drugs against (Woods et al. 1987). However, one of the limitations of the self-administration procedure is that it provides a confounded measure of reinforcing efficacy. For example, when a relatively large range of cocaine doses is studied in the self-administration paradigm, an inverted U-shaped dose-effect curve is obtained. That is, low and high doses of the drug maintain lower rates of responding than moderate doses. Since it has been determined that high doses of the drug are preferred (Johanson and Schuster 1975; Iglauer and Woods 1974), the descending limb of a self-administration dose-effect curve is not indicative of a decrease in reinforcing efficacy but is more likely the result of unconditioned (rate decreasing) drug effects (Woods et al. 1987). The ascending limb of the curve may also be partially influenced by unconditioned drug effects that result in nonspecific increases in motor activity. Thus, the dose-effect curve obtained from self-administration procedures is a function of both the reinforcing and response-rate-modulating effects of the drug.

Another factor present in the self-administration procedure is the discriminative stimulus functions of the drug. Drugs that are self-administered also result in interoceptive stimulus changes that allow an organism to accurately detect the delivery of a drug. However, the role of stimulus effects in drug self-administration has not been thoroughly investigated. Some questions that remain unanswered include the precise involvement of stimulus functions in the regulation of drug intake and the influence of stimulus effects in modulating the reinforcing efficacy of a drug. The self-administration procedure clearly involves several factors including reinforcing, rate-modulating, and stimulus effects of a drug, all of which may require independent investigation.

DRUG DISCRIMINATION

The drug discrimination paradigm has been used to evaluate the stimulus functions of cocaine. A typical drug discrimination procedure involves the use of an operant chamber containing two

response manipulanda. Prior to the observation period, subjects are trained to make one response if a drug is administered or the other if saline is delivered. Subjects quickly learn to detect and report the presence or absence of interoceptive drug-related stimuli. Once the animals are trained, the effects of other doses and other drugs, both alone and in combination with the training drug, can be evaluated. The degree to which they produce responding on the drug-associated option is used as an index for assessing stimulus properties.

Cocaine has robust stimulus properties, and animals are easily trained to discriminate cocaine from vehicle infusions (Woods et al. 1987). Studies investigating drugs that substitute for cocaine (i.e., result in responding on the cocaine-associated lever), or drugs that do not substitute for or block the discriminative stimulus effects of cocaine, provide data that implicate the activation of dopaminergic systems as one of the major factors responsible for the stimulus functions of the drug. However, the precise behavioral and neurobiological mechanisms involved in the discriminative stimulus functions of cocaine are still being intensely investigated (Woolverton and Kleven, this volume). Furthermore, an index of stimulus properties provides only an indirect assessment of abuse liability. The relationship between the stimulus effects and reinforcing effects of a substance is not clear. There are several classes of drugs that have stimulus properties and are not self-administered. Furthermore, drugs that substitute for cocaine in the drug discrimination task typically result in lower rates of self-administration or are sometimes not self-administered. Moreover, drugs that can attenuate the stimulus properties of cocaine may increase, have no effect on, or decrease self-administration. One final point about drug discrimination is that the procedure can result in a rate-independent assessment of stimulus properties. Doses of the drug that do not alter response rates result in drug-appropriate responding.

RATE DEPENDENCY

The observation that the ongoing rate of responding is an important determinant of the behavioral effect of a drug (Thompson et al. 1981) is one of the cornerstones of behavioral pharmacology. Studies on the effects of different classes of pharmacological agents on schedule-controlled behavior have continued to increase our understanding of drug action as well as behavioral mechanisms. The effects of cocaine on schedule-controlled behavior can be summarized as follows: (1) low doses capable of increasing motor activity have little or no effect on operant behavior; (2) intermediate doses increase low rates

of responding but produce less of an increase or have no effect on high rates; (3) larger doses increase low rates and decrease high rates; and (4) very large doses decrease responding regardless of rate. These effects have been observed using both simple and complex schedule contingencies and a variety of experimental subjects (Gonzalez and Goldberg 1977). A general conclusion is that, with few exceptions (Johanson 1978), regardless of how responding is engendered or maintained, cocaine results in comparable rate-dependent effects (Barrett 1976). These effects, however, are not specific to cocaine and are observed after the administration of other central nervous system stimulants.

A limited number of studies have investigated the effects of combinations of cocaine with other drugs on schedule-controlled behavior. Both the alpha$_1$-adrenergic antagonist prazosin (Tessel and Barrett 1986) and the neuroleptic chlorpromazine (Wilson and Schuster 1975) antagonize the rate-decreasing effects of cocaine, while atropine (Wilson and Schuster 1975) and ethanol (Aston-Jones et al. 1984) enhance the rate-decreasing effects of cocaine. The combination of diazepam and cocaine on schedule-controlled behavior can either attenuate or enhance the rate-increasing effects of diazepam alone, depending on the dose of cocaine administered (Ford et al. 1980).

Investigations of the effects of chlorpromazine in modulating the behavioral effects of cocaine epitomize the strength of a multifaceted behavioral approach. Chlorpromazine generally decreases responding regardless of the rate of responding or the event that is used to maintain responding. Furthermore, chlorpromazine can antagonize the rate-decreasing effects of cocaine on food-maintained responding (Wilson and Schuster 1975). Therefore, it is not surprising that the drug can alter cocaine self-administration (Gill et al. 1977; Herling and Woods 1980; Roberts and Vickers 1984). However, chlorpromazine can decrease the self-administration of low to moderate doses of cocaine (ascending limb of the dose-effects function), while not increasing the intake of higher doses (descending limb). In addition, chlorpromazine can also attenuate the discriminative stimulus effects of cocaine (Jarbe 1978). Thus, chlorpromazine attenuates the rate-decreasing effects of cocaine, decreases the self-administration of low to moderate doses, and attenuates the stimulus functions of the drug. Consequently, the effect of chlorpromazine on cocaine self-administration appears to be related to a decrease in both the reinforcing efficacy and stimulus effects of cocaine.

In summary, drugs that are abused, including cocaine, have at least three behavioral effects in common. They can modify the ongoing rate of behavior, they can have discriminative stimulus functions, and they are self-administered. The relative importance of these three behavioral effects in determining the abuse liability of cocaine requires further investigation. A neurobiological approach may provide the answers to some unresolved questions concerning the relative importance of these three behavioral properties as well as the neurobiological constituents of these behavioral effects.

NEUROBIOLOGICAL COMPONENTS OF COCAINE SELF-ADMINISTRATION

The neurobiological components of a phenomenon include: (1) the delineation of circuits and pathways involved in the event; (2) specification and localization of critical neurotransmitter receptors; and (3) evaluation of the influence of additional afferents and efferents. Examples of several procedures and the results that can be obtained concerning cocaine abuse are presented in several chapters in this monograph. This chapter will focus on the use of neurotoxin lesions to delineate the neurobiological aspects of the behavioral effects of cocaine.

Since a more complete presentation of this topic is included in the monograph (Koob and Hubner, this volume), a brief description and preliminary report using a procedure to simultaneously assess the neurobiological components of responding maintained by several reinforcers follows.

Rats were trained on a concurrent chained fixed-ratio (FR) 1, FR 9 (30-second timeout, 100-second limited hold) schedule using an operant conditioning chamber containing three retractable levers. Initially the three levers were available. A response on one of them (food, water, or drug) made the other two retract and set the contingency for the chosen lever as FR 9. Responding on the drug lever resulted in a 0.2-ml infusion of cocaine, responding on the food lever produced a 45-mg food pellet, and pressing the water lever resulted in presentation of a 0.1-ml water dipper.

The rats were first trained on the schedule using only the food and water levers with the cocaine lever retracted. They were implanted with chronic intravenous jugular catheters as well as bilateral guide cannulae, which were aimed into the central medial nucleus accumbens. Following recovery from surgery, the drug lever was extended

into the chamber, and a cocaine option was added to the food and water schedule. Since a preliminary study showed that rats given continuous access to cocaine would overdose themselves within 15 days (Dworkin et al. 1987), a restricted access condition to cocaine was imposed. The cocaine lever was available from 9 a.m. until 3 p.m., and the food and water levers were continuously available.

The initial dose of cocaine used to engender and maintain responding on the cocaine lever was 0.33 mg/infusion. The effects of substituting other doses of cocaine (0.08 to 0.83 mg/infusion) on responding maintained by the three reinforcers were determined (figure 1). Changing the cocaine dose that was available did not alter responding on either the food or water lever. However, the rate of self-administration showed an inverted V-shaped function. The highest rate of self-administration occurred at the 0.17 mg/infusion dose. Substitutions of saline for cocaine in the injection system resulted in a decrease in responding on the cocaine lever and no significant changes in responding on the food and water levers.

The effects of 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens on responding maintained by the three reinforcers were determined. The rats received two bilateral injections of 4 g in 0.5 l over 7.5 minutes after desipramine pretreatment (to protect noradrenergic neurons). The lesions were separated by 2 days. Following recovery from the lesions, the animals were again placed into the three-lever chamber and allowed to self-administer food, water, and cocaine. The lesions did not alter responding maintained by either food or water. However, the lesions flattened the dose-effect curve for cocaine self-administration by producing a specific effect on the ascending limb of the curve.

These investigations of the effects of 6-OHDA lesions on responding simultaneously maintained by food, water, and cocaine indicated that the lesions may have specifically attenuated the reinforcing effects of cocaine. The decrease in cocaine self-administration was not related to a nonspecific motor effect, since no effect was observed on food- or water-maintained responding. Furthermore, the lesions did not alter the unconditioned effects of cocaine on the descending limb of the self-administration dose-effect curve. Thus, the dopaminergic innervations of the nucleus accumbens destroyed by the injection of 6-OHDA appear to be involved in regulating cocaine intake as a result of direct modulation of the reinforcing effects of the drug.

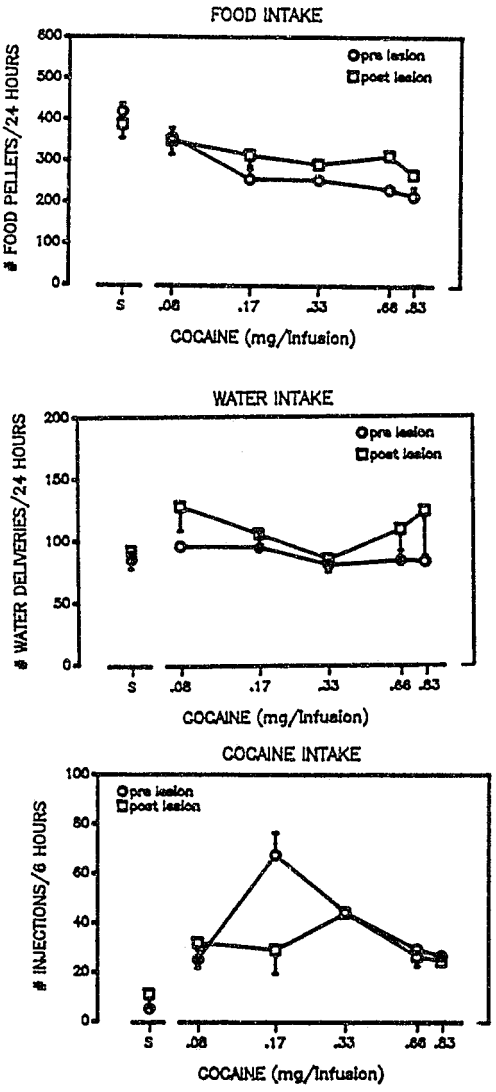

FIGURE 1. *The mean number of ratios completed on the food, water, and cocaine levers during a 24-hour period as a function of the dose of cocaine that was available for self-administration*

NOTE: Points collected before the lesions are indicated by the open circles, and the open squares indicate the effects of the lesions. The vertical lines show 1 SEM. The data presented are the means from three rats exposed to each dose for at least 3 days. Points above "S" show the effects of saline infusions.

NEUROBIOLOGICAL DETERMINANTS OF THE RATE-DEPENDENT EFFECTS OF COCAINE

In self-administration studies, the effects of neurotoxin lesions are not isolated from the influence of the reinforcing effects of the drug. Therefore, a second behavioral procedure was used to investigate the potential effects of the lesions on modifying the rate-dependent effects of cocaine. Rats were trained on what has become a standard operant baseline to investigate the rate-dependent effects of abused drugs. A multiple fixed-interval (FI), 4-minute FR, 15-response schedule of food presentation was used to maintain responding on a lever. At the start of the session, the first response after 4 minutes resulted in a food pellet delivery, and the next food pellet was delivered after the rat completed 15 responses. The rats were given 60 seconds from the end of the FI schedule or start of the FR to complete the schedule component (limited hold). If the schedule requirement was not completed by the end of the limited hold, the other component was scheduled. The schedule components alternated until 10 components of each were presented.

The schedule parameters used generated two different mean rates of responding. Responding maintained by the interval schedule was significantly lower than responding maintained by the ratio. However, cocaine resulted in similar response-rate effects on performance generated by both schedules. Doses of cocaine from 5.6 to 17 mg/kg had little or no effect on response rates, and the largest dose investigated generally decreased responding (figure 2).

After the prelesion dose-effect curves were determined, the rats were pretreated with desipramine and lesioned with 6-OHDA. Bilateral injections of 4 μg in 0.5 μl of the neurotoxin or its vehicle were delivered into the nucleus accumbens. The dose-effect curves were again determined following recovery from the lesion. The lesion did not result in any significant changes in the effects of cocaine on food-maintained responding. However, there was a trend for cocaine to result in a slightly greater decrease in responding after the lesion.

NEUROBIOLOGICAL COMPONENTS OF THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE

Rats were trained to discriminate cocaine (10 mg/kg IP) from saline using a standard two-lever procedure. Every 10th response (FR 10) on one of the levers (drug lever) resulted in the delivery of a food pellet if cocaine was injected before the session. Responding on the

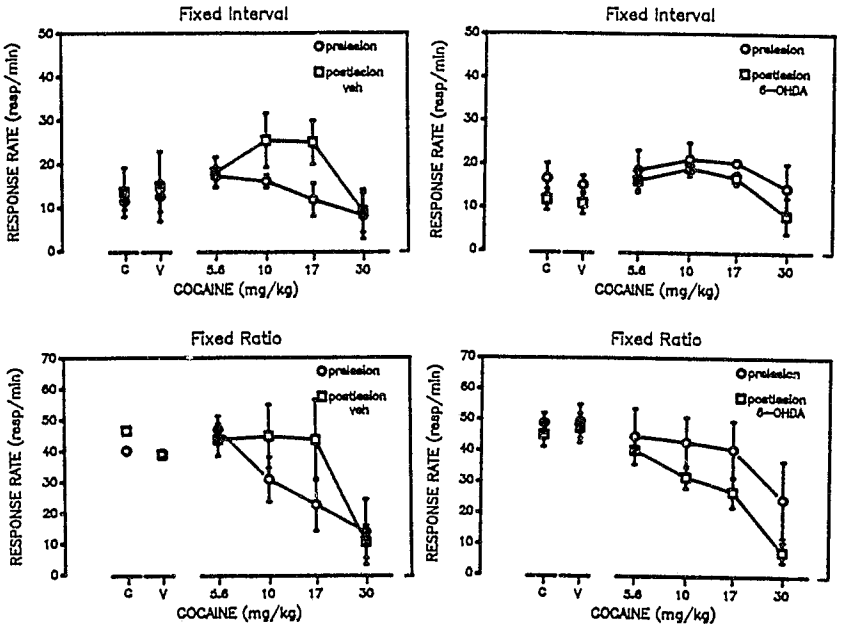

FIGURE 2. *Dose-response curves for the effects of cocaine on responding maintained by the multiple FI 4-minute. FR 15 schedule of food presentation*

NOTE: The circles indicate the effects of the drug before the lesion, and the squares show the postlesion effects. The points are the means from six rats, and the vertical lines indicate ±1 SD. The top panels show the effect on re-sponding maintained by the interval schedule, and the bottom panels indicate the effects of cocaine on responding maintained by the ratio schedule. The left and right panels show the effects of a vehicle and 6-OHDA lesion, respectively.

other lever (saline lever) resulted in reinforcement if the rat was pretreated with saline. Training was continued until the rats emitted the criteria of three or fewer responses on the incorrect lever before the first food presentation and more than 95 percent of the responses during a session occurred on the correct lever for five consecutive sessions.

The subjects were then tested using a multiple dosing procedure. The rats were injected with saline and immediately placed in the conditioning chamber. The session was started 1 minute following the injection and was terminated after 30 food pellets were obtained or 4

minutes of the session had elapsed. Five minutes after the first injection, the rats were injected with 0.82 mg/kg of cocaine, and a second session was started after 1 minute. This procedure was repeated with additional injections of 0.36, 0.50, 0.72, 1.0, 1.15, 2.1, and 3.0 mg/kg of cocaine 1 minute prior to the start of the third through ninth sessions. During testing sessions, responding on both levers was reinforced on an FR 10 schedule. Daily testing sessions were terminated when the subjects completed the first ratio on the drug lever after two consecutive injections. Training sessions were occasionally run between testing sessions.

Each rat was tested at least eight times before being lesioned with 6-OHDA or its vehicle. Bilateral injections of 6 μg in 0.4 ml over 7.5 minutes were made into the nucleus accumbens, following pretreatment with desipramine. Three days were allowed for recovery from the lesion. The animals were then tested again using the multiple dosing procedure for four consecutive testing sessions.

The multiple dosing procedure resulted in generalization gradients similar to those obtained when only single injections per day were evaluated (figure 3). All but one of the rats tested chose the saline lever after saline was injected and switched to the cocaine lever after the fifth cocaine injection. The vehicle lesion did not result in any significant changes in the discriminative stimulus properties of cocaine.

In contrast to sham animals, the 6-OHDA lesion severely attenuated the stimulus effects of cocaine. After the lesion, two of the subjects occasionally selected the cocaine lever after the first (saline) injection. In addition, these rats also completed the first ratio of the session on the cocaine lever after receiving low doses of the drug. Three rats, however, continued to choose the saline lever after saline injections and persisted in choosing the saline lever or showed no preference for either lever after additional injections of cocaine. Similar changes were seen in measures of the percent of responding on the drug lever during the testing sessions. Furthermore, neither the multiple injections nor the lesions produced any significant changes in the rate of responding during the sessions. Thus, the lesion produced a selective attenuation of the discriminative stimulus functions of the drug.

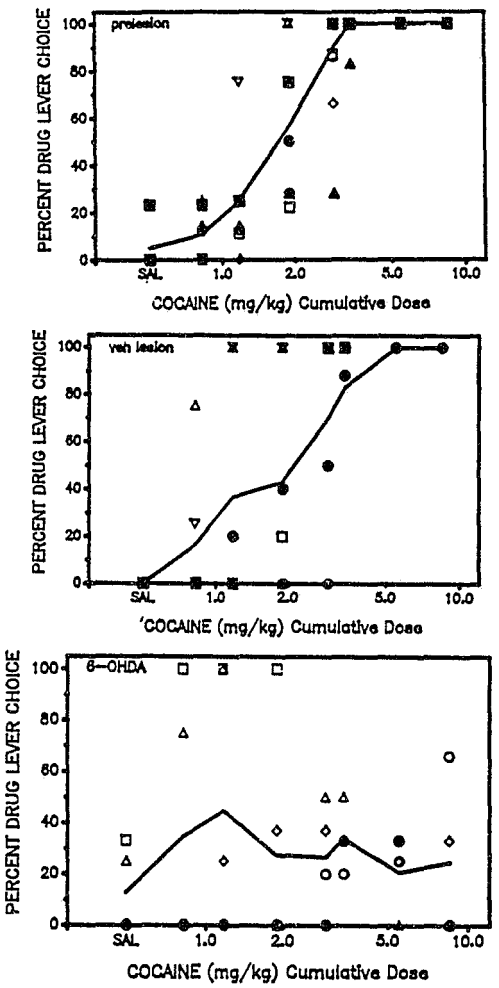

FIGURE 3. *Generalization gradients indicating the effects of consecutive doses of cocaine*

NOTE: Data presented indicate the percent of sessions during which the rats completed their first ratio on the drug lever. The data collected from each rat are indicated by the individual points, and the lines indicate the mean of data collected from all subjects. The top left panel shows the gradient obtained before the lesion. Data presented in the middle panel were collected after a vehicle lesion, and the bottom panel shows the effects of the neurotoxin lesion.

CONCLUSION

The dopaminergic system innervating the nucleus accumbens is clearly involved in modulating both the reinforcing and discriminative stimulus functions of cocaine. Selective lesions of this system attenuate cocaine self-administration while having little or no effect on food or water intake. Furthermore, rates of responding maintained by food and water presentations and the effects of cocaine on food- and water-maintained responding are not affected by the lesion. Cocaine self-administration involves the reinforcing, rate-modulating, and discriminative stimulus effects of the drug. It appears, however, that the reinforcing effect of the drug is more a function of the stimulus functions of the drug than its effects on ongoing rates of responding. Schedule-controlled behavior, however, does provide an excellent screen to evaluate the potential for a drug to attenuate self-administration. Both chlorpromazine and benzodiazepines alter the effects of cocaine on schedule-controlled behavior and attenuate cocaine self-administration. Evaluations of the effects that pharmacologic and neurobiologic perturbations have on the behavioral effects of cocaine increase our understanding of the behavioral and neurobiological aspects of drug abuse.

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Intracranial Cocaine Self-Administration

Nick E. Goeders

INTRODUCTION

The nonmedical use of cocaine has continued to increase during the last decade, while the use of other illicit drugs has steadily declined since 1979. While the neurobiological mechanisms potentially involved in the complex processes mediating cocaine reinforcement have been under vigorous examination, they have not yet been conclusively elucidated. The intravenous self-administration paradigm has been the most frequently employed laboratory tool used to investigate cocaine reinforcement, since this route most closely parallels human self-administration. Neuronal pathways and circuits that may be involved in cocaine reinforcement processes can be identified by assessing the effects of specific neurotransmitter antagonists or neurotoxin lesions on self-administration as described elsewhere in this volume. However, the distinct brain sites responsible for the initiation of neuronal activity in these reinforcement-related pathways and circuits following the response-contingent administration of cocaine may be difficult if not impossible to identify following systemic delivery. With systemic self-administration, it is difficult to conclude accurately whether pharmacological or neurobiological treatments specifically affect the reinforcing properties of the drug or whether they affect other processes such as rate of responding, conditioned responses to the drug, ability to discriminate the presence of the drug, or changes in heart rate, blood flow, or drug metabolism and elimination. These and other factors can nonspecifically influence self-administration and may result in potentially erroneous conclusions. Intracranial self-administration (ICSA) offers an exciting alternative procedure for providing valuable information that can be used to complement intravenous self-administration data for a better understanding of the neurobiological mechanisms of drug reinforcement. With ICSA, the

completion of the appropriate response results in the delivery of the drug directly into a discrete brain region. If interactions of the drug with receptors localized within the injection locus result in the activation of neuronal systems associated with reinforcement, then these intracranial microinjections should maintain responding and, therefore, circumvent many of the problems inherent in systemic drug delivery.

Although the full potential for ICSA has only recently been realized, the idea of the direct self-administration of a drug into brain tissue dates back almost 30 years (Olds and Olds 1958; Olds 1962). Intraventricular self-administration of drugs (Gustafson and Pickens 1975; Amit et al. 1976; Belluzi and Stein 1977; Tortella and Moreton 1980; Brown et al. 1979) has been demonstrated as has the direct intracranial self-administration of opioids, psychomotor stimulants, and putative neurotransmitters. Among the opioids, morphine is self-administered into the lateral hypothalamus (Stein and Olds 1977; Olds 1979), the ventral tegmental area (Bozarth and Wise 1980; Bozarth and Wise 1981), and the nucleus accumbens (Olds 1982), while D-ala^2-methionine enkephalinamide, a stable enkephalin analog, maintains ICSA into the lateral hypothalamus (Olds and Williams 1980), and methionine-enkephalin, an endogenous ligand for delta opiate receptors, is self-administered into the nucleus accumbens (Goeders et al. 1984). With respect to putative neurohumors, neurotensin supports ICSA into the ventral tegmental area (Glimcher et al. 1983), while cholecystokinin maintains ICSA into the nucleus accumbens (Hoebel and Aulisi 1984). Dopamine is self-administered into the nucleus accumbens (Guerin et al. 1984; Dworkin et al. 1986), and preliminary data suggest that glutamate maintains ICSA into the ventral pallidum and preoptic area (Goeders et al. 1987). The psychomotor stimulants amphetamine and cocaine are self-administered into the mesolimbic/mesocortical dopaminergic system. Cocaine maintains ICSA into the medial prefrontal cortex (Goeders and Smith 1983), while amphetamine is self-administered into the orbitoprefrontal cortex of rhesus monkeys (Phillips and Rolls 1981) and the nucleus accumbens of rats (Monaco et al. 1981; Hoebel et al. 1983). The remainder of this chapter will focus on what has been learned about the central mechanisms of cocaine reinforcement using the intracranial self-administration approach.

DRUG DELIVERY SYSTEMS

ICSA is an elegant methodology for demonstrating that a discrete brain region is involved in the initiation of reinforcing neuronal

activity following the response-contingent administration of a drug. By restricting the delivery of the drug to potentially reinforcement-relevant receptor populations, this procedure may minimize many of the peripheral effects resulting from systemic drug administration. However, it is important to recognize some of the limitations that may be involved in the use of this technique and in the interpretations of the data obtained. To engender and subsequently maintain responding, the substance must be delivered directly to the reinforcement-relevant receptor populations with minimal delay. An ideal drug delivery system is one that rapidly dispenses a small, reproducible volume under moderate pressure. Large injection volumes (i.e., greater than 500 nl) can result in nonspecific local tissue damage at the injection site and backflow of the injectate (Olds 1962). Diffusion of the injectate is directly related not only to the infusion volume but also to the rate of infusion (Myers 1974; Myers and Hoch 1978; Routtenberg 1972). If there is a significant diffusion from the site of injection, the precise identification of the reinforcement-relevant receptors may be severely compromised. To avoid mechanical damage to tissue and significant diffusion from the injection site, incredibly small injection volumes (i.e., less than 1/2,000 ml) must be accurately delivered.

Drug delivery systems similar to those used for intravenous self-administration have also been utilized for ICSA. These systems consist of a microsyringe controlled by a motor-driven syringe pump, which is activated for a predetermined duration each time that the animal completes the appropriate response. While these delivery systems are relatively accurate when used with restrained animals, their use for discrete, small-volume intracranial injections in relatively unrestrained subjects may lead to inaccurate drug delivery due to mechanical effects of the tubing used to connect the microsyringe to the injection cannula. Electrolytic microinfusion systems using chemitrodes (Criswell 1977) circumvent many of the problems experienced with small-volume drug delivery.

An electrolytic drug delivery system consists of an airtight drug reservoir, containing two electrodes, which is attached to an injection cannula that mounts directly on a guide cannula on the animal's head. Microinfusions are produced by passing a direct current between the two electrodes, resulting in the evolution of hydrogen gas which, in turn, increases the pressure in the reservoir and forces a reproducible volume of drug solution out through the injection cannula. The amount of solution infused is proportional to the current intensity and duration. With the electrolytic systems, movement does not

affect drug delivery, and continuity between responding and reinforcer presentation is achieved. Electrolytic microinfusion systems were used in all the cocaine intracranial self-administration experiments described below.

The first series of experiments determined the accuracy and dependability of the electrolytic microinfusion system for cocaine ICSA (Goeders et al. 1985) by using tracer amounts of radiolabeled cocaine mixed with unlabeled cocaine. The injection cannula was placed into fresh brain tissue, and varying intensities of either an injection current or a quiescent current were passed between the electrodes. The tissue was then solubilized, and the radioactivity was determined with liquid scintillation spectrophotometry. This calibration demonstrated that an injection current of 200 μA with a 6-μA quiescent current delivered reliable 100-nl microinfusion volumes (± 7 percent) with no leakage between injections.

Next, the extent of diffusion of the drug from the injection site was determined. Rats received 4 μl of ^{3}H-cocaine in 40 infusions of 100 nl each delivered over 5.5 seconds on a random time, 4-minute schedule (similar to the maximum rate of self-administration) into a self-administration site in the medial prefrontal cortex (Goeders and Smith 1985). The animals were sacrificed by total immersion in liquid nitrogen 30 seconds following the final injection. Ninety-three percent of the radioactivity was recovered within 1 mm^3 of the cannulae tips, suggesting that the reinforcing actions of cocaine are probably localized within the target area in the medial prefrontal cortex and not at distal sites as a result of drug diffusion. A cyclic voltagram demonstrated that the cocaine was not electrochemically altered by the current intensities produced by the electrolytic microinfusion system. These initial studies demonstrated that the electrolytic microinfusion system is the ideal drug delivery system for investigating intracranial cocaine self-administration.

MAPPING INTRACRANIAL COCAINE SELF-ADMINISTRATION

Although the effects of cocaine on neurotransmission are complex, the primary neurochemical action appears to be an inhibition of biogenic amine neurotransmitter uptake into presynaptic nerve endings. Results obtained from intravenous self-administration experiments have demonstrated a primary role for the mesolimbic/mesocortical dopaminergic neuronal system in cocaine reinforcement (Koob and Hubner, this volume). Therefore, initial behavioral experiments were designed to investigate whether intracranial

injections of cocaine would maintain self-administration into various target sites located within the mesolimbic dopaminergic system (Goeders and Smith 1983). Rats were stereotaxically implanted unilaterally with guide cannulae into the nucleus accumbens, ventral tegmental area, or medial prefrontal cortex. The animals were housed on a reversed 12-hour light-dark cycle and tested for intracranial self-administration at the start of their active cycle. Sessions were terminated either after 8 hours or after 40 injections were delivered to minimize potential cumulative cytotoxic effects. A red light above the response lever was illuminated at the start of the session, and each response in the presence of the red light resulted in a 5.5-second infusion of 100 nl of the drug. During the infusion, the red light was extinguished, a white light was illuminated, and a tone was presented. Each microinfusion was followed by a 30-second timeout period during which both stimulus lights were extinguished, and responses were monitored but had no scheduled consequences. Concentrations of 0 to 5,000 pmol of the drug dissolved in artificial cerebrospinal fluid were tested in the three brain regions.

Microinjections of cocaine into the nucleus accumbens or ventral tegmental area did not maintain responding at any concentration tested, but microinfusions into the medial prefrontal cortex resulted in a rapid acquisition of lever pressing. These results were surprising in light of intravenous self-administration data, which suggest a major role for the nucleus accumbens in cocaine reinforcement processes. Therefore, a second group of animals implanted with cannulae into the nucleus accumbens was trained to lever-press before being retested for intracranial self-administration (Goeders and Smith 1987). These rats were originally tested for intracranial self-administration; they were then food-deprived to 80 percent of their *ad lib* weights and allowed to respond on a variable interval, 30-second schedule of food reinforcement in the same experimental chamber. When stable baselines of responding were observed (approximately 1 week), the food magazine was removed from the chamber, and the microinjection system was secured to the rat's head for ICSA testing. However, even after lever-pressing training, these rats did not self-administer cocaine, suggesting that cocaine does not initiate reinforcing neuronal activity by its response-contingent application into the nucleus accumbens.

However, all rats with cannulae implanted into the medial prefrontal cortex self-administered cocaine, with an average of six behavioral sessions required to engender stable rates of self-administration at the training dose of 50 pmol/100 nl infusion. When lever pressing

stabilized for at least three consecutive sessions at this dose, dose-response curves were determined by increasing or decreasing the drug concentrations.

Self-administration was dose related, with maximal rates of responding obtained with 50 to 90 pmol/infusion. Dose-response relationships were unique for each animal, with responding maintained over either narrow or wide ranges in different subjects. Large changes in drug intake often occurred after increasing or decreasing the concentration by as few as 5 or 10 pmol. Rates of self-administration declined as the dose was either increased or decreased from optimum levels. An empirical measure of the patterns of self-administration was therefore employed to analyze the self-administration data (Goeders et al. 1986). The interinfusion intervals were counted separately in four 15-minute divisions (0 to 15 minutes, 15 to 30 minutes, 30 to 45 minutes, and 45 to 60 minutes), a frequency distribution was generated for each session, and relative frequencies were calculated. The significance of difference in the mean relative frequency distributions of the interinfusion intervals for the various treatment conditions was evaluated with a two-factor randomized analysis of variance followed by Tukey's all pairwise comparisons among treatment means.

A two-lever discrimination procedure was used to demonstrate that self-administration resulted from the reinforcing properties of cocaine and not from a nonspecific increase in motor activity (Goeders and Smith 1983). For these lever-reversal experiments, an identical but inactive lever was installed on the opposite wall of the chamber, with responses being recorded but having no scheduled consequences. When responding stabilized, active contingencies were alternated between the right and left sides of the chamber. In this two-lever choice procedure, responding rapidly increased on the active lever. When the active and inactive levers were reversed, the rats switched responding to the new active manipulandum. Extinction (i.e., the substitution of vehicle for cocaine) resulted in a loss of this preference, and the animals responded at equally low rates on both levers. During reconditioning, when cocaine microinfusions were again made available, the animals rapidly increased responding primarily on the active lever. Since responding occurred significantly more often on the active manipulandum during several reversals, these data suggest that the self-administration did not result from a generalized, nonspecific behavioral stimulant action of cocaine. Furthermore, in some animals, the response requirement was gradually increased from

1 to 10 lever presses per microinfusion, and the animals correspondingly increased their rates of responding to maintain similar interinfusion intervals and patterns of self-administration. These data demonstrate that the response-contingent delivery of cocaine into the medial prefrontal cortex does serve as a reinforcer.

NEUROPHARMACOLOGY

In addition to being classified as a psychomotor stimulant, cocaine also possesses local anesthetic properties. Therefore, the next series of experiments tested whether the local anesthetic properties of cocaine were responsible for the ICSA behavior (Goeders et al. 1986). Rats were trained to self-administer cocaine into the medial prefrontal cortex, and dose-response curves were determined for each animal. Equimolar concentrations of lidocaine HCl and nine times equimolar concentrations of lidocaine HCl were then substituted for the optimum dose of cocaine (the dose resulting in the highest and most stable drug intake). Lidocaine was used in these experiments because, unlike other local anesthetics (e.g., procaine, chloroprocaine), it does not appear to have reinforcing properties. However, this substitution resulted in rates of drug intake similar to vehicle, with patterns of responding resembling those seen with either a very low dose of cocaine or the vehicle. These data suggest that the reinforcing properties of cocaine are unrelated to its local anesthetic effect.

The neurotransmitter receptors involved in the intracranial self-administration of cocaine were investigated using specific receptor antagonists. Rats were trained to self-administer cocaine into the medial prefrontal cortex, and dose-response curves were determined for each animal as described above. Equimolar concentrations of the D-2 dopaminergic receptor antagonist sulpiride, the D-1 dopaminergic receptor antagonist SCH 23390, the beta-noradrenergic receptor antagonist dl-propranolol hydrochloride, or the muscarinic cholinergic receptor antagonist atropine sulfate were coadministered in a random order with the optimum dose of cocaine. If an attenuation of self-administration was observed, the concentration of cocaine was doubled, and the effects of the antagonist were again determined. If no change in intake occurred, then the concentration of the antagonist was doubled and the effects again determined. Sulpiride significantly decreased drug intake to 47 percent of baseline levels and generated patterns of self-administration (i.e., mean relative frequency distributions) consistent with those seen when the concentration of cocaine was decreased from optimum levels. This effect persisted for

two sessions, but drug intake returned to baseline by the third posttreatment session. Doubling the concentration of cocaine did not alter the sulpiride effect, suggesting a noncompetitive action at separate receptor sites. The administration of the other antagonists did not alter drug intake directly. Atropine had no effect on rates or patterns of self-administration during the session it was delivered but significantly decreased drug intake during the first posttreatment session, with patterns of responding consistent with those seen with higher doses of cocaine. These effects dissipated by the second posttreatment session and were not altered by doubling the concentration of atropine. Similar results were observed with equimolar and twice-equimolar concentrations of SCH 23390. Propranolol, however, did not affect the rates or patterns of self-administration at any concentration tested.

These results suggest that D-2 dopaminergic receptors in the medial prefrontal cortex appear to be excitatory to cocaine reinforcement processes, since equimolar concentrations of sulpiride reduced self-administration to vehicle levels with patterns of responding consistent with decreasing the cocaine dose. Cocaine and sulpiride may act at different receptor sites, since the attenuation of self-administration after D-2 receptor blockade was not reversed by increasing the cocaine dose. On the other hand, the D-1 dopaminergic receptor antagonist SCH 23390 and the muscarinic cholinergic receptor antagonist atropine had either no effect or minimal effects on intracranial self-administration. However, the blockade of these receptors resulted in a delayed attenuation of drug intake, with patterns of responding resembling those seen with an increased dose of cocaine (i.e., a potentiation of cocaine-initiated neuronal activity). Beta-noradrenergic systems in the medial prefrontal cortex may not be involved in cocaine reinforcement.

6-HYDROXYDOPAMINE LESIONS

These data suggest that the initiation of cocaine reinforcement processes results, in part, from direct or indirect interactions of the drug with D-2 dopaminergic receptors in the medial prefrontal cortex. To determine whether the reinforcement-relevant receptors are localized on presynaptic dopaminergic terminals or on postsynaptic neurons, the self-administration site was lesioned with the neurotoxin 6-hydroxydopamine (Goeders and Smith 1986). Rats were implanted with guide cannulae into the medial prefrontal cortex and trained to intracranially self-administer cocaine as described above. When dose effect analyses were completed and stable baselines of drug intake

obtained, animals were pretreated with desmethylimipramine to inhibit uptake into noradrenergic neurons followed by unilateral 6-hydroxydopamine injections into the medial prefrontal cortex at the self-administration site. In postlesion self-administration sessions, various concentrations of cocaine were tested for self-administration in each rat, and the rates and patterns of intake at each concentration were compared with those obtained prelesion.

The lesion resulted in a 45 percent decrease in the content of dopamine in the medial prefrontal cortex, while the levels of the other biogenic amine neurotransmitters serotonin and norepinephrine were not affected. However, there may have been a hyperactivity of serotonergic neuronal activity on the nonlesion side, since the content of serotonin was increased 85 percent compared to control animals. No changes were observed in neurotransmitter content in the nucleus accumbens, suggesting that the lesion was localized to neurons in the medial prefrontal cortex.

The 6-hydroxydopamine lesion at the site of self-administration reduced drug intake to vehicle levels and flattened the dose-response curves for all animals. Prelesion rates of self-administration did not return during the 6 to 8 weeks postlesion that the behavior was monitored, and responding could not be reinitiated by either increasing or decreasing the cocaine concentration. However, the substitution of dopamine (300 pmol) for cocaine resulted in a rapid reacquisition of lever pressing almost to prelesion baseline levels, with rates and patterns of responding similar to those maintained by the high or optimum dose of cocaine prior to the lesion. This dopamine self-administration was probably mediated through post-synaptic D-2 dopaminergic receptors, since blockade with the coinfusion of sulpiride reduced intake to vehicle levels. Serotonergic receptors do not appear to be involved, since the substitution of 300 pmol of this neurotransmitter would not maintain responding in the lesioned animals. The involvement of serotonin cannot be ruled out, however, since different concentrations of the neurotransmitter were not tested in these animals. In summary, these experiments demonstrated that dopaminergic innervations are necessary for ICSA of cocaine in the medial prefrontal cortex. The 6-hydroxydopamine lesions that damaged presynaptic dopaminergic terminals eliminated the primary mechanism for cocaine-induced initiation of reinforcing neuronal activity. Postlesion response-contingent microinfusions of dopamine appeared to activate postsynaptic neuronal systems in a manner analogous to the delivery of cocaine prelesion.

NEUROTRANSMITTER TURNOVER

A final set of experiments used neurotransmitter turnover procedures to investigate whether the response-contingent delivery of cocaine into the medial prefrontal cortex results in the activation of neuronal activity mediated through other brain regions. Rats were implanted unilaterally with cannulae into the right medial prefrontal cortex. One group of rats was allowed to self-administer cocaine, while another group served as a yoked-vehicle control. Depression of the lever by a rat in the self-administration group simultaneously delivered a microinfusion of artificial cerebrospinal fluid to the yoked-vehicle rat contained in a chamber similar to the self-administration chamber. Depressions of the lever by the yoked-vehicle animals were counted but had no scheduled consequences, although all stimulus changes were identical. After stable baselines of responding were observed at the optimum concentration of cocaine in the self-administration group, all rats were implanted with jugular catheters using previously reported methods (Weeks 1962; Weeks 1972). The catheter was sealed at the end and enclosed in a light-weight aluminum backpack (Lane et al. 1982). Following a 1-week recovery from surgery, the rats were allowed to self-administer cocaine at the optimum concentration until stable rates of drug intake were obtained. Two days prior to sacrifice, the patency of each jugular catheter was determined by injecting 100 μl of methohexital. An immediate behavioral depression indicated that the catheter was functional. The catheters were resealed, and the animals were returned to their home cages.

Immediately prior to the start of the experimental session, one of the self-administration animals and its yoked-vehicle control received 0.5 mCi of L-[U-^{3}H] tryptophan and 1.0 mCi of L-[2,6-^{3}H]-tyrosine injected in 100 μl of heparinized saline through the jugular catheters. Two timepoints (60 and 90 minutes) were used to determine turnover following the administration of the radioactive precursors. These timepoints were chosen because they have been demonstrated to be on the linear portion of the decay in radioactivity curves for the biogenic amine neurotransmitters (Smith and Lane 1983). Self-administration rats and yoked-vehicle rats were simultaneously sacrificed at the appropriate pulse times by total immersion in liquid nitrogen in combination operant conditioning/liquid nitrogen immersion chambers (Smith et al. 1977). The heads were warmed in -18 °C cryostat, and the brains were removed and cut in the coronal plane into 500-μm serial sections. The right and left sides of the medial prefrontal cortex and nucleus accumbens were microdissected

from these coronal sections. The contents of norepinephrine, dopamine, serotonin, tyrosine, and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured using high-pressure liquid chromatography with electrochemical detection, and specific radioactivity was determined using liquid scintillation spectrophotometry (Co et al. 1982).

Turnover rates were calculated (Smith et al. 1982) and expressed as pmol mg protein^{-1} hr^{-1}.

No significant differences in neurotransmitter content were observed between treatment groups in either brain region (tables 1 and 2). Increased levels of tyrosine and DOPAC were seen in the right medial prefrontal cortex compared to the left in both self-administration rats and yoked-vehicle rats (table 1), suggesting that dopamine utilization is greater on the right side of the brain compared to the left. Significant changes in neurotransmitter turnover were observed in both medial prefrontal cortex and nucleus accumbens as a result of the response-contingent intracranial delivery of cocaine, but only on the side of the brain containing the injection cannula. At the site of self-administration in the right medial prefrontal cortex, decreases in the utilization of dopamine (-87 percent) and serotonin (-90 percent) and increases in the utilization of norepinephrine (435 percent) were found (table 3). In contrast, the turnover rate for dopamine was increased (128 percent), norepinephrine decreased (-69 percent), and serotonin unchanged in the right nucleus accumbens (table 4).

The turnover rates for dopamine in the left nucleus accumbens of both treatment groups and the right nucleus accumbens of the yoked-vehicle rats were consistent with the values reported from other investigations (Smith et al. 1982). However, the utilization of dopamine was significantly increased in the right nucleus accumbens of the self-administration rats, suggesting that dopaminergic activity in this structure was enhanced. The medial prefrontal cortex sends efferent connections to a number of subcortical structures including the ventral tegmental area (Leonard 1969) and nucleus accumbens (Beckstead 1979; Walaas 1981). Cocaine administration into the medial prefrontal cortex may result in the activation of an ipsilateral descending neuronal system projecting to one or more of these brain areas. This, in turn, could result in the activation of the dopaminergic innervations of the nucleus accumbens with increased synthesis and release of the neurotransmitter. These data are in agreement with investigations of the effects of 6-hydroxydopamine lesions of the nucleus accumbens on intravenous cocaine self-administration, which suggest that the dopaminergic innervations of

TABLE 1. *Content of dopamine (DA), norepinephrine (NE), serotonin (5-HT), tyrosine (TYR), and dihydroxyphenylacetic acid (DOPAC) in right and left medial prefrontal cortex of rats self-administering cocaine into the medial prefrontal cortex and in yoked-vehicle rats*

Medial Prefrontal Cortex	Treatment Group	DA	NE	5-HT	TYR	DOPAC
Right Side	ICSA	17.6±5.2	14.4±4.2	29.6±5.6	570.7±242*	16.5±9.0^{+}
	Yoke	16.2±7.0	16.9±5.1	27.9±3.3	602.9±243*	18.6±13.3*
Left Side	ICSA	15.1±1.8	16.6±2.4	32.4±7.5	350.7±80	5.3±2.5
	Yoke	16.3±6.2	17.0±3.1	30.6±5.8	344.1±77	5.4±2.1

NOTE: Values are means ± standard deviations for n=8 in each treatment group, measured in pmol mg protein^{-1}. Significant differences between the right and left sides for each treatment group determined with Student's t-tests were: *$p<0.05$; $^{+}p<0.01$.

TABLE 2. *Content of dopamine (DA), norepinephrine (NE), serotonin (5-HT), tyrosine (TYR), and dihydroxyphenylacetic acid (DOPAC) in right and left nucleus accumbens of rats self-administering cocaine into the medial prefrontal cortex and in yoked-vehicle rats*

Nucleus Accumbens	Treatment Group	DA	NE	5-HT	TYR	DOPAC
Right Side	ICSA	321.0±75.9	14.7±4.8	40.0±8	488.8±135	54.0±10.5
	Yoke	393.0±104.8	14.9±4.1	43.7±8.5	471.3±131	77.4 ± 24
Left Side	ICSA	277.6±44	20.3±8.2	36.6±10.4	455.0±122	44.3±11.1
	Yoke	297.3±72	12.3±4.7	38.6±9.9	488.0±146	59.2±15.5

NOTE: Values are means ± standard deviations for n=8 in each treatment group, measured in pmol mg protein^{-1}

TABLE 3. *Turnover rates of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) concurrently measured in right and left medial prefrontal cortex of rats self-administering cocaine into the medial prefrontal cortex and in yoked-vehicle rats*

Medial Prefrontal Cortex	Treatment Group	DA	NE	5-HT
Right Side	ICSA	4.2±3.0^{+}	30.1±8.2^{+}	6.2±4.0^{+}
	Yoke	31.7±6.4	1.4±0.3	61.0±21.4
Left Side	ICSA	9.4±9.1	8.2±7.5	31.9±8.1
	Yoke	10.1±5.3	18.7±8.2	16.3±11.8

NOTE: Values are means ± standard deviations for n=8 in each treatment group, measured in pmol mg protein^{-1} hr^{-1}. Significance of the differences determined with Student's t-tests were: $^{+}p<0.01$.

TABLE 4. *Turnover rates of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) concurrently measured in right and left nucleus accumbens of rats self-administering cocaine into the medial prefrontal cortex and in yoked-vehicle rats*

Nucleus Accumbens	Treatment Group	DA	NE	5-HT
Right Side	ICSA	318.0±44^{+}	3.1±1.1*	10.1±1.6
	Yoke	139.7±43	10.2±0.3	19.6±15.2
Left Side	ICSA	152.5±77	ND	13.2±2.1
	Yoke	122.5±52	ND	19.6±12.2

NOTE: Values are means ± standard deviations for n=8 in each treatment group, measured in pmol mg protein^{-1} hr^{-1}. Significance of the differences determined with Student's t-tests were: $^{*}p<0.02$; $^{+}p<0.01$. ND, data not collected.

this structure are involved in cocaine reinforcement (Roberts et al. 1980).

CONCLUSIONS

The response-contingent delivery of cocaine into the medial prefrontal cortex initiates reinforcing neuronal activity directly through the presynaptic inhibition of the reuptake of dopamine and indirectly through the postsynaptic activation of dopaminergic receptors by increased synaptic concentration of the neurotransmitter.

These effects may result in the downregulation of dopamine utilization in the medial prefrontal cortex and the initiation of neuronal activity in endogenous reinforcement circuits. The various cell bodies and fiber connections within these pathways may each be involved in the mediation of cocaine reinforcement processes, with a loss of function at any point in the circuit detrimental to the full expression of these effects. While the nucleus accumbens and ventral tegmental area did not support intracranial self-administration, these data do not exclude the possibility that other neurotransmitter systems in other brain regions might maintain this behavior, especially since the intravenous administration of cocaine results in a much more potent reinforcing stimulus than is produced through the intracranial route. It would be naive to assume that the magnitude of reinforcement produced by the systemic delivery of the drug results from interactions in a single brain area, especially when the intracranial administration of the drug can, at best, result in a small stimulus change in the organism. The actions of cocaine at multiple initiation sites are probably responsible for the degree of reinforcement observed following intravenous presentation. A more thorough understanding of cocaine reinforcement processes can be obtained through the identification of these loci and the integration of these data with those obtained using intravenous self-administration procedures.

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Psychomotor Stimulant vs. Local Anesthetic Effects of Cocaine: Role of Behavioral Sensitization and Kindling

Robert M. Post and Susan R.B. Weiss

INTRODUCTION

This paper focuses on behavioral sensitization and pharmacological kindling, two distinct effects of cocaine, which appear to be related to cocaine's separate effects as a psychomotor stimulant and local anesthetic. Both of these effects increase in intensity following repeated, intermittent administration of a constant drug dose. Behavioral sensitization, increased responsivity to the locomotor-stimulating or stereotypy-inducing effects of cocaine, can be produced by relatively low doses of cocaine. Pharmacological kindling, repeated administration of subconvulsive doses of cocaine eventually resulting in the development of full-blown seizures, occurs with higher doses and is similar to that produced by other local anesthetics such as lidocaine. Thus, it would appear that the unique properties of cocaine as both a psychomotor stimulant and a local anesthetic carry with them the liability of inducing, upon repetition, increased behavioral pathology and convulsive responsivity. These two mechanisms, demonstrated in a variety of preclinical animal laboratory studies, may have clinical relevance for the human cocaine user who tends to become involved with repeated administration of either low, high, or escalating doses. It is possible that the psychomotor stimulant properties of cocaine, which are associated with behavioral sensitization, may play a role in the evolution and development of cocaine-induced dysphorias and paranoid psychoses like those reported for amphetamine, while the local anesthetic properties of cocaine may be more closely associated with the late development of cocaine-induced panic attacks, seizures, and the attendant potential lethality.

We will also emphasize an important role for environmental context and conditioning in the development of cocaine-induced behavioral sensitization. Initial studies of the possible neural substrates mediating this effect will be presented. In some paradigms, cocaine-induced behavioral sensitization may be attributable to conditioning variables, while, as doses are increased and repeated more frequently, a context-independent behavioral sensitization can also become evident; i.e., sensitization will occur independent of conditioning variables. At the highest doses, pharmacological kindling can ensue, an effect that may also be independent of conditioning mechanisms. If the present analysis is relevant to the human situation, it would suggest that there are inherent toxicities to repeated cocaine use that can become increasingly manifest even if the abuser of cocaine were never to escalate his cocaine dose.

COCAINE-INDUCED BEHAVIORAL SENSITIZATION

Elsewhere, we have reviewed some of the characteristics of cocaine-induced behavioral sensitization in detail (Post and Contel 1981). Briefly, there appears to be a long-lasting change in behavioral responsivity, which is thought to involve alterations in dopaminergic function, perhaps in the mesolimbic system. Behavioral sensitization is dose-dependent (Shuster et al. 1977), occurs at lower doses in females than in males, and may be, in part, dependent upon intact vasopressin function (Post and Contel 1981). Cocaine-induced behavioral sensitization tends to show cross-sensitization to other psychomotor stimulants, especially amphetamine, and also to show cross-sensitization to a variety of environmental stressors (Antelman et al. 1980; Kalivas et al. 1986; Kalivas et al. 1988; Duffy and Kalivas, in press; DuMars et al. 1988). The interval between injections appears important, with greater time between injections associated with more robust sensitization up to a certain point (which has not been adequately delineated).

A particular focus of this manuscript will be the role of environmental context and conditioning in cocaine-induced behavioral sensitization. As previously demonstrated (Post et al. 1981), repeated administration of cocaine in the same environment leads to marked increases in locomotor hyperactivity and stereotypy, while pretreatment in one environment and testing in a dissimilar environment leads to lesser degrees of sensitization. We have recently attempted to reproduce the environmental context finding in a more expeditious fashion. We have adopted a 1-day pretreatment paradigm in which animals are injected with cocaine (40 mg/kg IP) or saline on day 1

and rechallenged with cocaine (10 mg/kg IP) on day 2 (Weiss et al. 1988). In this paradigm, as illustrated in figure 1 (left panel), animals show a substantial cocaine-induced hyperactivity in response to the 40 mg/kg injection, while saline-pretreated controls show only minimal degrees of activity. A third group of animals was also used in this study; they received saline in the test chamber and the same dose of cocaine (40 mg/kg) in their home cage following the experimental session. In response to the challenge dose of cocaine (10 mg/kg) on day 2, only those animals that received cocaine in the context of the test cage showed notable degrees of behavioral sensitization, and animals that received equal doses of cocaine in the context of the home cage did not differ from saline-pretreated controls (figure 1, right panel). Thus, it would appear with this single high-dose paradigm that all of the cocaine-induced sensitization is related to a conditioned process.

The effects are dose related. Pretreatment with cocaine (10 mg/kg IP) on day 1 does not lead to behavioral sensitization to rechallenge on day 2. However, if cocaine (10 mg/kg IP) is repeated for several days, significant degrees of sensitization are evident by 4 to 7 days (Post et al. 1981). In this paradigm, the behavioral sensitization also appears to be environmental-context-dependent. With this repeated-dose paradigm, significant increases in response to a saline challenge are also evident in sensitized animals, while this is not apparent with the single-dose sensitization method.

If high-dose cocaine pretreatment (40 mg/kg IP) is repeated for 3 days, cocaine-induced behavioral sensitization shifts from a predominance of locomotor hyperactivity as an endpoint to more predominant stereotypy. With this shift, there is also a change from sensitization involving exclusively a conditioning component to one in which the stereotypic behavior demonstrates a sensitization in a fashion that is independent of environmental context. These dose-repetition relationships are summarized in table 1. We would suggest the possibility that this shift might involve a change in neural substrates with the nucleus accumbens and amygdala being involved in the environmental-context-dependent sensitization and striatal and other extrapyramidal dopaminergic substrates, more typically associated with stereotypic behaviors, mediating the context-independent cocaine-induced sensitization. Finally, as illustrated in table 1, with high doses, cocaine's local-anesthetic mechanisms are brought "on line" and pharmacological kindling results, perhaps requiring additional neuronal pathways (Post et al. 1984a).

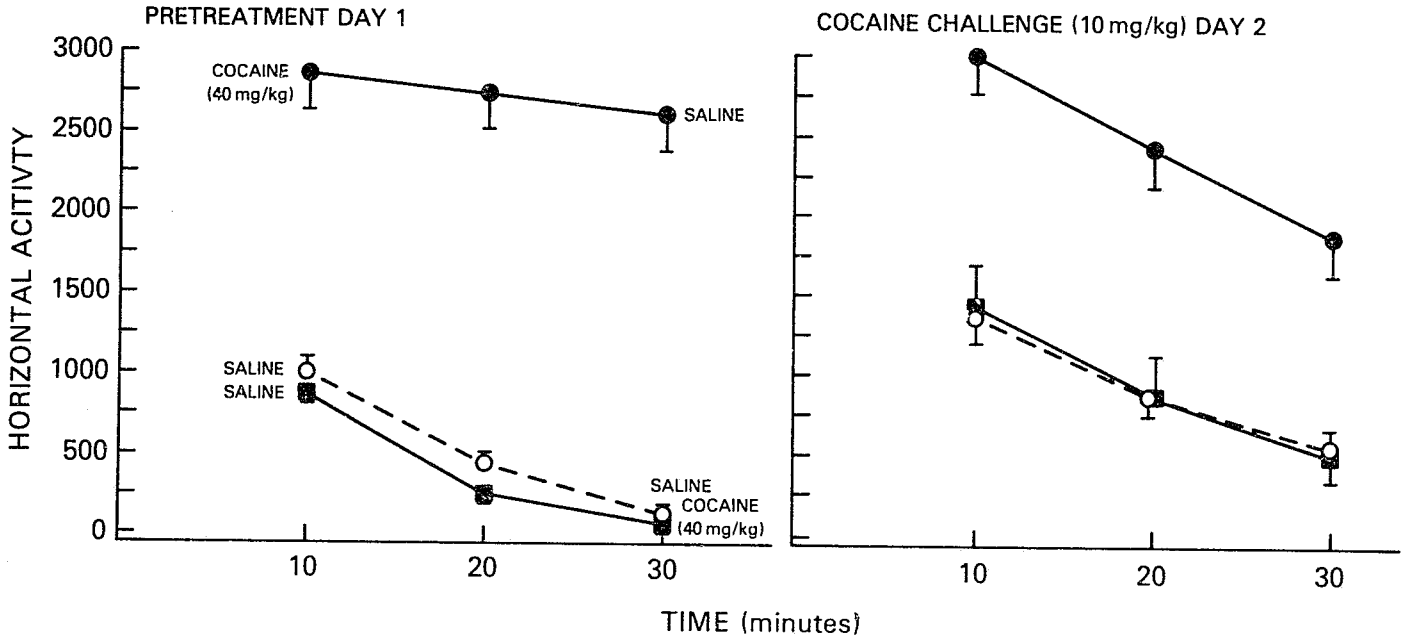

FIGURE 1. *Cocaine-induced behavioral sensitization depends on environmental context*

NOTE: Left panel: Horizontal locomotor activity is illustrated on the ordinate and time on the abscissa for three groups of rats (n=10/group) receiving the following day-1 pretreatments: cocaine in the test cage and saline in the home cage (filled circles); saline in the test cage and cocaine in the home cage (filled squares); saline in both cages (open circles). Right panel: Horizontal activity is illustrated for these three groups following a cocaine challenge (10 mg/kg). Only the rats that

TABLE 1. *Long-term effects of cocaine: Function of dose and number of repetitions*

COCAINE		EFFECT								
Number of Injections	Dose mg/kg i.p.	Behavioral Sensitization Duration	Activity Context Dep.	Activity Context Indep.	Stereotypy Context Dep.	Stereotypy Context Indep.	Saline Conditioning	Sensitization Neuroleptic Independent	Seizure Kindling	Death
↑↑↑	COC_{65}								++	++
x10 days	COC_{160} subcut. (K. Gale)	++		++		++		++		
↑↑↑↑↑↑↑↑↑↑	COC_{10}	++ months	++	0			++			
↑↑↑	COC_{40}	+	0	++	++	++	±			
↑	COC_{40}	++ days	++	0	0	0	0	0		
↑ ↑ ↑	COC_{20}	0 0	0	0	0	+				
↑ ↑ ↑	COC_{10}	0 0	0	0	0	0				
↑	COC_{10}	0 0								

NOTE: Size and number of arrows indicate dose of cocaine and number of administrations. COC = cocaine and dose in mg/kg administered once daily IP, except in the study of Gale, when doses were subcutaneous. 0 = no effect; ± = equivocal; + = moderate effect; ++ = marked or definite effect. Effects of cocaine increase and are more persistent with dose and number of repetitions. They also become less dependent on environmental context and conditioning. The highest doses may be associated with kindling of seizures and their associated lethality. Thus, effects shift from behavioral sensitization to kindling as the dose is increased.

In the single-day cocaine-induced behavioral sensitization paradigm, we have attempted to further elucidate variables related to the conditioning phenomenon. We have demonstrated that the degree of

similarity of the pretreatment and test environments is a critical factor in determining the degree of behavioral sensitization (Weiss et al. 1988). Animals pretreated and tested in the same environment show the most robust degree of behavioral sensitization, although animals pretreated in a Plexiglas cage that is very similar to the activity-monitored cage also show significant degrees of behavioral sensitization. When the initial place where animals display their cocaine-induced hyperactivity is very different from that of the test environment, such as one involving a wire cage in a different room, there is no significant cocaine-induced behavioral sensitization.

Manifestation of cocaine-induced hyperactivity or stereotypy on day 1 is required for behavioral sensitization to become evident on rechallenge on day 2. That is, when we pretreated animals with neuroleptics or high doses of benzodiazepines in order to block day 1 cocaine-induced hyperactivity, no sensitization was evident upon rechallenge on day 2 (Weiss et al. 1988). This effect was evident at both moderate (0.2 mg/kg) and high (0.5 mg/kg) doses of haloperidol.

However, while these doses were sufficient to block the development of sensitization when haloperidol was administered prior to the day-1 pretreatment, they were not sufficient to block the expression of sensitization when they were administered only prior to the day-2 test dose (figure 2). That is, once cocaine-induced hyperactivity had been manifest on day 1, moderate to high doses of neuroleptics were insufficient to block the differential effects of this prior cocaine experience on subsequent cocaine-induced hyperactivity. These data replicate and extend those of Beninger and Herz (1986), Beninger and Hahn (1983), and Tadokoro and Kuribara (1986).

Elsewhere (Post and Weiss 1988), we have discussed in detail the interpretation of these results with respect to neuroleptic nonresponsiveness in some psychotic states. Basically, once the sensitized behaviors have become established, neuroleptics may no longer be capable of reversing these behaviors. To the extent that behavioral sensitization accounts for some aspects of cocaine-induced syndromes, one might postulate that these components would be less responsive to neuroleptics than in instances where acute cocaine-induced behavioral pathology is induced.

In contrast to neuroleptics, specific 6-hydroxdopamine lesions (with desmethylimipramine pretreatment) lesions of the nucleus accumbens and amygdala appear capable of blocking cocaine-induced sensitization while leaving the degree of day-1 cocaine-induced hyperactivity intact

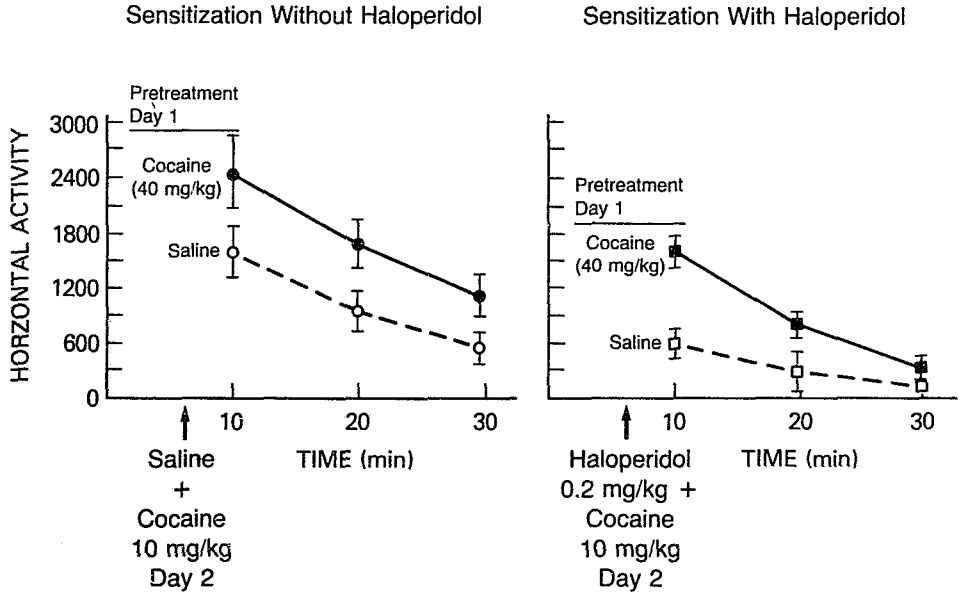

FIGURE 2. *Cocaine-induced behavioral sensitization: Haloperidol does not block its expression*

NOTE: Haloperidol does not block the expression of sensitization to cocaine once it is induced. Group means and standard errors for horizontal activity are plotted over time in animals challenged with saline prior to cocaine (10 mg/kg) (left panel) or haloperidol (0.2 mg/kg) prior to cocaine (10 mg/kg) on day 2 (right panel). Animals that received cocaine (40 mg/kg) pretreatment on day 1 are indicated in the filled symbols; those given saline, open symbols. The left panel illustrates the magnitude of cocaine-induced sensitization without haloperidol; the right panel shows the persistent sensitization effects with haloperidol.

(Post et al. 1987b). While nucleus accumbens dopamine depletion of 60 to 65 percent was not sufficient to block cocaine-induced hyperactivity in response to the day-1 injection with cocaine (40 mg/kg IP), these animals were not different in their response on day 2 from saline-pretreated controls.

Electrolytic lesions of the amygdala also blocked the development of cocaine-induced behavioral sensitization, while lesions of the dorsal hippocampus increased baseline hyperactivity but left the differential effect of prior cocaine intact. We have subsequently replicated the effect of amygdala lesions, demonstrating that both electrolytic lesions and selective depletion of dopamine in the amygdala (achieved by 6-hydroxydopamine and desmethylimipramine cotreatment) were sufficient to block cocaine-induced behavioral sensitization (Post et al. 1987b). Lesions of the ventral hippocampus or cerebellum were not effective in blocking sensitization in this paradigm.

Thus, preliminary data would suggest that dopaminergic pathways involving the nucleus accumbens and amygdala may be involved in the mediation of the conditioned cocaine sensitization, possibly affecting the coding of the experience of the prior cocaine-induced hyperactivity and/or its impact on subsequent behavior. Gold and associates (in press) have also suggested that the nucleus accumbens is involved in the conditioned component of amphetamine-induced behavioral sensitization. However, in their studies, more complete lesions of the nucleus accumbens were achieved, which also blocked the unconditioned amphetamine-induced behavior. Clearly, further studies are needed in order to elucidate the precise anatomical pathways and neurochemical substances involved in the conditioned component of cocaine-induced behavioral sensitization.

PHARMACOLOGICAL KINDLING

Goddard and associates (1969) first named the process "kindling," in which repeated electrical stimulation of the brain at subthreshold currents eventually led to the development of major motor seizures. A critical requirement for the development of kindling is intermittent stimulation, since continuous stimulation or stimulation at intervals of only several minutes never leads to the production of seizures. With repeated, intermittent electrical stimulation, there is increasing duration, spread, and complexity of afterdischarges, which culminate in the appearance of major motor seizures. Once these have been achieved, there seems to be a permanent change in excitability lasting for 6 months to 1 year or longer. Moreover, if kindled seizures are

induced a sufficient number of times, a condition of spontaneity occurs in which seizures occur in the absence of exogenous electrophysiological stimulation (Pinel 1981; Pinel 1983). Early evidence suggested that a parallel process of "pharmacological kindling" attributable to cocaine's local anesthetic properties might occur with repeated high-dose drug administration (Post et al. 1975; Post and Kopanda 1976; Post 1977).

In order to more systematically assess this possibility, we administered the pure local anesthetic lidocaine and observed a kindlinglike timecourse at doses of 60 to 65 mg/kg daily. In this paradigm, approximately 40 percent of animals eventually developed seizures to what was previously a subconvulsant dose of drug. Moreover, the seizures resembled those achieved by amygdala kindling, with involvement of head, trunk, and forepaws, with rearing and falling. Electrophysiological spiking was also evident in the amygdala. Deoxyglucose studies of regional metabolic activity revealed prominent increases in glucose utilization in hippocampus and amygdala or perirhinal structures achieved during lidocaine-induced seizures (Post et al. 1984a). Following sufficient repetitions of lidocaine-induced seizures, spontaneous seizures developed parallel to the phenomenon of spontaneity in electrical kindling.

As reported by Eidelberg and associates (1963), cocaine also induces prominent spiking in related limbic structures. When a dose of cocaine (65 mg/kg) that is equipotent to lidocaine's local anesthetic effects is administered on a once-daily basis, more than 50 percent of the animals show seizures on day 1. Moreover, all of the animals administered this dose of cocaine experienced seizures and died by day 3 of the study, even though a majority of animals tolerated repeated lidocaine-induced seizures for many weeks or months. Thus, it would appear that cocaine's unique combination of local anesthetic and psychomotor stimulant properties make the local anesthetic seizures more lethal than those achieved by the pure local anesthetic lidocaine.

We have observed several interesting effects of the anticonvulsant carbamazepine on local anesthetic kindling. Chronic carbamazepine treatment, using a diet containing 5.0 g carbamazepine/kg food, almost completely blocks the development of lidocaine-kindled seizures, but is without effect on completed kindled or high-dose lidocaine seizures, although acute administration of the benzodiazepine diazepam blocks kindling development and completed seizures. Similarly, chronic carbamazepine is highly potent in inhibiting the

development of cocaine-kindled seizures (figure 3). In contrast to its effects on high-dose lidocaine seizures, carbamazepine decreases the incidence of high-dose cocaine seizures and their associated lethality. This effect is even more robust if a lower dose of cocaine is used to more clearly demonstrate pharmacological kindling. A roughly similar timecourse of seizure evolution can be achieved by cocaine (40 mg/kg) compared with lidocaine (65 mg/kg), such that none of the animals demonstrates seizures or dies on day 1, although some 80 percent of the animals experience seizures by 2 weeks of treatment. In this instance, chronic carbamazepine remarkably decreases cocaine-induced seizure development (from 80 to 25 percent) and decreases lethality from 50 to 5 percent by day 16.

Notably, however, acute carbamazepine in doses ranging from 15 to 50 mg/kg is without effect on either lidocaine- or cocaine-kindled or high-dose seizures (figure 4). Furthermore, pretreatment with repeated acute injections of carbamazepine (15 mg/kg IP) is also without effect on the development of cocaine or lidocaine seizures and may actually increase the lethality associated with the local anesthetic seizures (figure 5). In contrast, acute IP administration of the benzodiazepine diazepam will decrease both pharmacological kindling development to the local anesthetic lidocaine as well as completed lidocaine-kindled seizures. Thus, chronic but not acute carbamazepine treatment appears to produce specific biochemical effects that may be of use in the prevention of seizure development.

EFFECTS OF PURE LOCAL ANESTHETICS IN MAN

It is thus apparent that repeated administration of high doses of local anesthetics are capable of inducing pharmacological kindling in animals. Before further considering the possible implications of this phenomenon in humans, should high enough doses of cocaine be self-administered, it is important to consider what is known about the subjective physiological effects of more moderate doses of pure local anesthetics administered to man. Although in one study the effects of nasally insufflated lidocaine were mistaken for those of cocaine in experienced cocaine users (Van Dyke et al. 1979), lidocaine is generally adequately discriminated from cocaine (Fischman et al. 1983a), and this local anesthetic is not self-administered by animals (Balster, this volume). In contrast, procaine is self-administered by animals, and its effects are more often confused with those of cocaine in clinical experiments with sophisticated stimulant abusers (Fischman et al. 1983b).

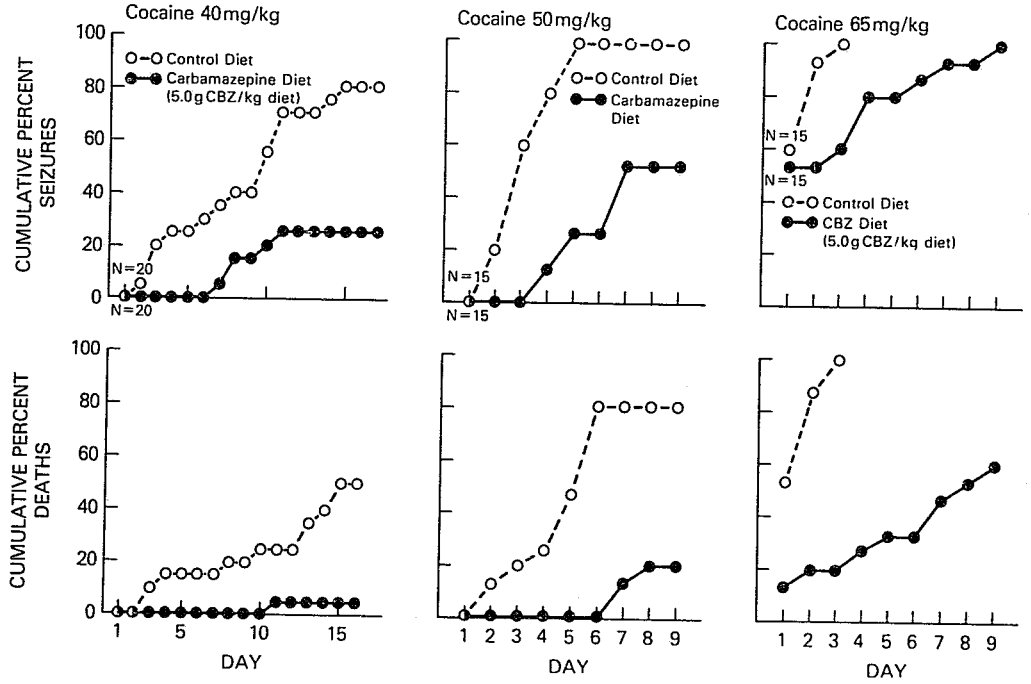

FIGURE 3. *Chronic carbamazepine prevents the development of cocaine-kindled seizures and mortality*

NOTE: Rats were placed on a control or carbamazepine-containing diet for 4 days prior to the studies and were kept on these diets throughout the period of cocaine injections. Cocaine was administered in doses of 40, 50, or 65 mg/kg. Carbamazepine was effective in inhibiting the cocaine-induced seizures and lethality.

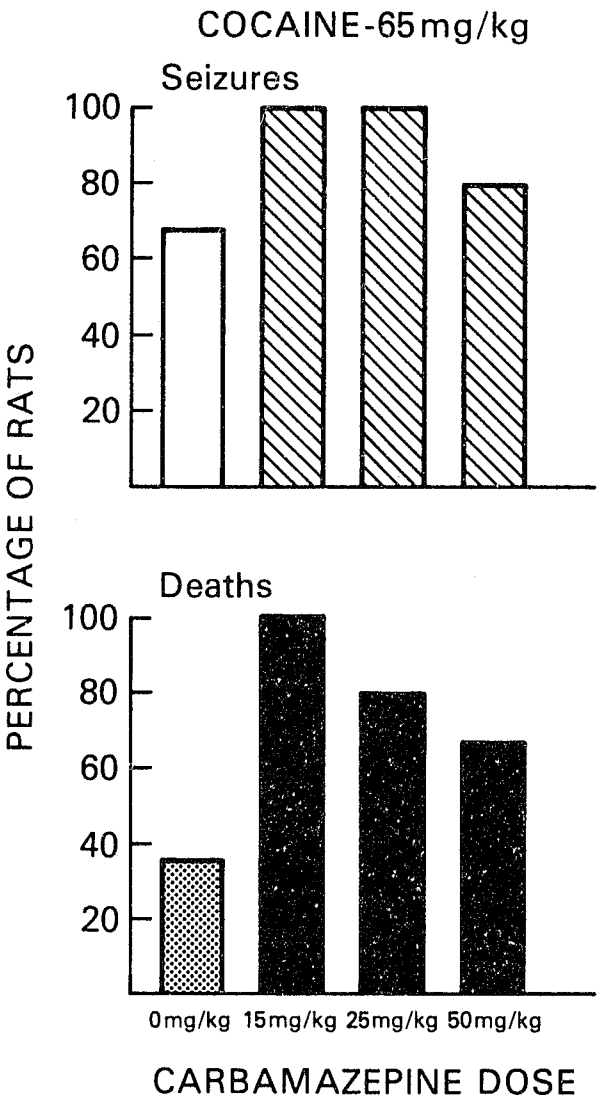

FIGURE 4. *Lack of effect of acute carbamazepine on cocaine-induced seizures and lethality*

NOTE: Carbamazepine was administered IP, 15 minutes before cocaine, in doses of 0, 15, 25, or 50 mg/kg in an attempt to block acute high-dose (65 mg/kg) cocaine seizures and lethality. No anticonvulsant effect was seen at any of these doses. Cocaine-induced lethality either was not affected or was exacerbated by these acute doses.

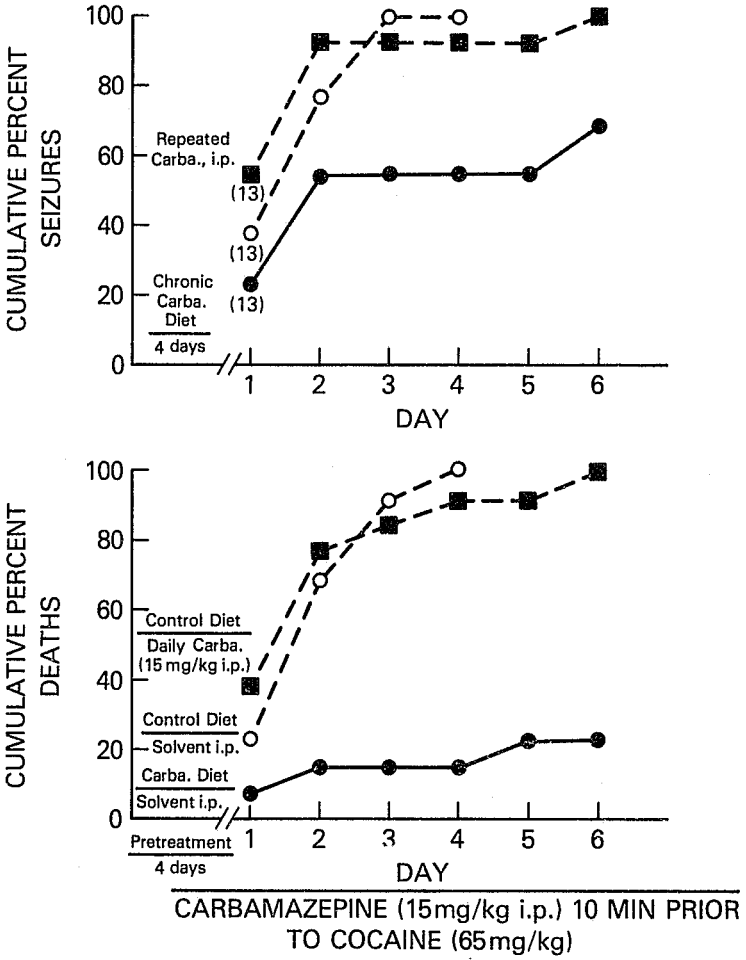

FIGURE 5. *Pretreatment with chronic carbamazepine in diet (not repeated injection) is required to block cocaine seizures and lethality*

NOTE: Rats were treated with chronic carbamazepine (diet), IP-injected carbamazepine (15 mg/kg), or vehicle for 4 days prior to cocaine and IP carbamazepine (15 mg/kg) throughout the study. This regime of repeated IP carbamazepine treatment did not decrease the response to high-dose cocaine, in contrast to the chronic oral dosing.

We administered ascending doses of procaine to naive normal volunteers and psychiatrically ill patients with diagnoses of primary affective illness or borderline personality disorder (Kellner et al. 1987). Procaine has about one-quarter the local anesthetic potency of cocaine or lidocaine, and was used because of its rapid metabolism and safety in man. In doses ranging from .46 to 2.30 mg/kg IV, we found dose-related effects of IV procaine on psychosensory and cognitive distortions in a variety of sensory modalities. In contrast, there were marked variations in the degree of affective response, ranging from euphoria to profound dysphoria. Increases in fast EEG frequencies in the beta range from 26 to 45 Hz were evident selectively over the temporal cortex. The degree of activation of these fast frequencies was correlated with the degree of dysphoric reaction in the first 21 subjects studied. Procaine was also associated with an endocrine profile of increases in plasma ACTH and cortisol as well as prolactin, but not growth hormone. It is of interest that preclinical studies have indicated that cocaine, procaine, and lidocaine all induce release of corticotropin releasing factor (CRF) in hypothalamic preparations *in vitro* and that carbamazepine blocks this cocaine-induced release of CRF (Calogero et al. 1987).

CLINICAL IMPLICATIONS

Based on the procaine data, we would suggest that some of the acute effects of cocaine normally associated with its psychomotor stimulant properties could, in fact, be related to its local anesthetic effects. These might include mood lability and, in some instances, euphoria, profound anxiety and dysphoria, tinnitus, sensory distortions, hallucinatory-like phenomena, and even seizures should the dose be high enough. Based on the extrapolation from the pharmacological kindling effects that have been demonstrated in preclinical studies with repeated administration, we would suggest that some of the chronic effects of cocaine could also be attributed to its local anesthetic properties, including sensitization to bizarre behavior, seizure sensitization (pharmacological kindling), interictal irritability and aggression (particularly in subjects who have shown local anesthetic-induced seizures), cognitive impairment, and even panic attacks. It was strikingly unexpected to find that some 50 percent of the first 500 patients calling the Cocaine Hotline to report adverse psychological effects reported experiencing cocaine-induced panic attacks (Washton and Gold 1984).

Based on the previous clinical observations of local anesthetic dysphoria and preclinical data on local anesthetic kindling, we would

suggest that this extraordinarily high incidence of cocaine-induced panic attacks might be related to cocaine's local anesthetic rather than its psychomotor stimulant properties. We are not aware of a similar high incidence of amphetamine-induced panic attacks being reported by any of the many investigators reporting epidemiological data on the psychological consequences of amphetamine or by the laboratories reporting psychological effects of amphetamine administered under more controlled conditions. These background data, along with our data (Kellner et al. 1987) show that many patients experience the local anesthetic procaine, even in low doses, as extremely dysphoric, and do so in proportion to the degree of EEG activation over the temporal lobe, further support the contention that the local anesthetic effects of cocaine might be related to these dysphoric experiences. If we further factor in the possible pharmacological kindlinglike effects occurring at a subconvulsant level, it is possible that repeated cocaine-induced activation of temporal lobe structures, as has been documented to occur in man (Ervin et al. 1969), may be sufficient to induce a kindlinglike increase in physiological and behavioral responsivity eventually leading to the development of panic attacks.

The temporal characteristics of such a proposed course are evident in a case observed by Uhde, Geraci, and colleagues in a clinic for panic-anxious patients at the National Institute of Mental Health (Post et al. 1987a). A 23-year-old single white male reported 3 years of essentially continuous daily use of cocaine by the intranasal route prior to his experience of his first cocaine-induced panic attack, immediately following cocaine insufflation. Over the next 5 months, he continued to experience cocaine-related panic attacks (approximately five times weekly); in each instance, these occurred in immediate association with cocaine use. However, after these many repetitions of cocaine-induced panic attacks, he developed a spontaneous panic attack and assumed that his cocaine-related panic attacks had led to this autonomous occurrence. He thus gave up his daily cocaine use, but despite this, continued to experience intermittent and unpredictable spontaneous panic attacks. These became associated with high levels of generalized anxiety and increasing agoraphobia, leading to his eventually becoming housebound. These spontaneous panic attacks improved with treatment with not only such traditional agents as imipramine, but also with the anticonvulsant carbamazepine and, later, alprazolam.

Thus, it would appear that these panic attacks followed a kindling-like timecourse in that they did not occur with the first of many

repetitions of cocaine administration, but then began to occur on a regular basis with cocaine use, and finally, like the stage of spontaneity observed in both electrical and pharmacological kindling, began to occur in the absence of local anesthetic administration. It is also possible that various aspects of behavioral sensitization and conditioning, demonstrated with subconvulsant doses in animals, are relevant to the progressive development of such symptomatology. Moreover, it is difficult to assess the exact dose of a drug that is administered in uncontrolled street-use settings, as reported in this case study, and whether a relatively constant dose was used over time.

The likelihood of adverse consequences related to behavioral sensitization and kindlinglike effects may be further enhanced because of gradual escalations in actual cocaine doses over time. Since there is ample evidence that tolerance or tachyphylaxis can develop to cocaine's euphoria-inducing properties (Fischman et al. 1976), increasingly high doses might be employed by the user in an attempt to recapture and/or maintain the euphoria or positive affective state. This may, in fact, be a pharmacological impossibility, as suggested by the recent studies of Sherer and colleagues (1986) indicating that, even with maintenance of high steady-state cocaine levels with continuous IV infusion in volunteers, increasingly dysphoric elements and paranoia begin to emerge. In the face of dose escalation, one might eventually achieve blood levels of cocaine high enough to induce toxic local anesthetic effects. Then, with repeated administration of even the same subconvulsant doses, the habitual user might, in addition, be at increased risk for the induction of seizures.

If the findings from the preclinical studies reviewed are relevant to the clinical situation, it would appear that cocaine-induced behavioral and psychological toxicities may, in fact, grow with repetition of the same dose over time, just as they do in cocaine-induced behavioral sensitization. Moreover, in some circumstances, powerful conditioned phenomena appear to be engaged by cocaine, although at higher doses and with greater numbers of repetitions, context-independent behavioral sensitization also occurs. Our conditioning findings in rodents are of greater interest now that a variety of conditioned phenomena have been reported in man. For example, greater degrees of paranoia are experienced when cocaine is used in the place where it is habitually administered rather than when it is used in novel environments (D.L., personal communication, 1986). Gawin and associates (1986) reported numerous vignettes of conditioned cues inducing

relapses, cocaine craving, or withdrawal syndromes. Similarly, Childress et al. (1987) reported that pictures of cocaine-related paraphernalia were powerful conditioned cues in producing cocaine craving in abstinent former cocaine users, similar to that described with opiates. Craving could be systematically extinguished using principles of learning theory. These findings are also of interest in relationship to the report of Hinson and Poulos (1981) that cocaine-induced behavioral sensitization in rats could, likewise, be deconditioned with repeated injections of saline.

It is also noteworthy that Stein and Belluzzi (1986) have reported conditioning with cocaine at the level of the single cell. The cell was "rewarded" with cocaine whenever it increased its firing rate. Compared with pseudoconditioned controls, which were administered cocaine at random intervals, the cocaine-conditioned cells "learned" to increase their firing rates to a remarkable and significant degree. Thus, cocaine-induced conditioned phenomena appear to be robust at the level of the single cell (Stein and Belluzzi 1986), the rodent (Post et al. 1981; Hinson and Poulos 1981), the cat (Ellinwood and Kilbey 1980), and, finally, man (Gawin et al. 1986; Childress et al. 1987).

Perhaps specific therapeutic strategies aimed at the powerful nature of cocaine-induced behavioral sensitization and its associated conditioned cues may be helpful in the long-term treatment of cocaine users. Preclinical studies also suggest the need for considerable caution in relationship to potential clinical utility of pharmacological interventions. For example, neuroleptics may or may not be effective depending on the phase of development of cocaine-induced symptoms that the user is experiencing. Not only might the neuroleptic nonresponsiveness of cocaine-induced behavioral sensitization provide a model for neuroleptic refractoriness in some psychotic states clinically, but it should also lead to a search for pharmacological interventions that may be effective on this phase.

The local anesthetic effects of cocaine and their associated liability for pharmacological kindling appear to add a danger beyond that experienced by users of the pure psychomotor stimulants such as amphetamine, methamphetamine, methylphenidate, and related compounds. Moreover, pharmacological interventions, such as acute carbamazepine, do not reverse cocaine-induced seizures (figures 4 and 5), limiting any potential clinical utility of an acute approach strategy. Carbamazepine also does not block the euphoria of stimulants such as methylphenidate (Meyendorff et al. 1985). However, based on our preclinical analysis of local anesthetic-related

kindlinglike effects, we would predict that chronic carbamazepine might be capable of blocking the development of cocaine-induced panic attacks and seizures clinically. Since carbamazepine does not block cocaine-induced hyperactivity acutely or the development of behavioral sensitization (Post et al., in press), evidence of the efficacy of this compound in the development of cocaine-induced panic would clearly suggest that cocaine-induced panic attacks were more closely related to a kindlinglike rather than a sensitizationlike mechanism.

Thus, it is likely that repeated cocaine administration may carry the danger of increased behavioral and physiological toxicities, even with use of the same dose over time. Behavioral sensitization (figure 1) and kindling paradigms (figure 3) that focus on possible mechanisms for this effect may help direct us to the neuroanatomical substrates involved (e.g., the nucleus accumbens and amygdala), and eventually lead to the possibility of new psychological and pharmacological treatment interventions. Further study of the evolution of cocaine-related syndromes not only may be of value in developing new treatments for cocaine-related behavioral and physiological toxicities in man, but also may provide important clinical and preclinical models for a variety of endogenous psychiatric syndromes and their evolution in man, including affective disorders, panic attacks, and paranoid schizophreniform states. It is also increasingly important for the potential and often naive user of cocaine to be made aware of the large variety of extremely adverse psychological and physiological consequences of acute and, especially, chronic use of this drug, effects that may not be at all evident early in the course of cocaine use. Since cocaine has such powerful reinforcing effects, which are extraordinarily difficult for many users to resist, it is critical to educate the public regarding potential extreme adverse consequences of repeated cocaine use.

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Genetic Differences in Responses to Cocaine

Frank R. George and Steven R. Goldberg

INTRODUCTION

Cocaine is an effective reinforcer and at present a widely used drug of abuse. It produces marked physiological effects (e.g., changes in heart rate and blood pressure) as well as behavioral effects, including euphoria and increased locomotor activity. There is, however, significant individual variability in cocaine uptake and metabolism, as well as in behavioral responses to cocaine. This suggests that genetic factors may play an important role in determining the extent of responses to cocaine. Although the pharmacology and biochemistry of this drug have been widely studied, little is known about the contribution of genotype in determining response to this increasingly abused substance.

Studies with other abused drugs such as ethanol and, to a lesser extent, opiates have repeatedly demonstrated that genetic factors play an important role in regulating the effects of these drugs (Broadhurst 1979; Crabbe and Belknap 1980; McClearn and Rogers 1961). One widely used measure of drug effects in animal models is locomotor activity. Genetic differences in locomotor response to ethanol and opiates, as well as to xanthines and barbiturates, have been shown (Ritz et al. 1981; Shuster et al. 1975; Oliverio and Castellano 1974; Logan et al. 1986; Suzuki et al. 1987).

In addition to locomotor and other acute responses to drug challenge, genetic factors could also play a role in regulating drug-seeking behavior. The use of inbred strains and selectively bred lines in studies on ethanol (McClearn and Rodgers 1961; Rodgers and McClearn 1962; Eriksson 1968; Li et al. 1979; Eriksson and Rusi 1981; Tampier et al. 1981; Elmer et al. 1986; Ritz et al. 1986; George 1987),

has provided valuable information about the contribution of genetic factors to ethanol drinking.

There are very few reports of studies on genetic differences in intake of nonethanol drugs. The existing data, all of which involve studies on home cage drinking of opioid solutions, do suggest that large population differences may exist with regard to drug-seeking behavior (Nichols and Hsiao 1967; Carroll et al. 1986; George and Meisch 1984). A different approach to the analysis of drug-seeking behavior involves self-administration studies using operant techniques. Under certain conditions, drugs from several pharmacological classes can act as positive reinforcers (Deneau et al. 1969; Weeks 1962; Schuster and Thompson 1969; Goldberg 1976; Spealman and Goldberg 1978; Griffiths et al. 1979; Meisch 1984; Young and Herling 1986). Recently, several reports have shown significant genetic influences on ethanol self-administration (Ritz et al. 1986; Elmer et al. 1986; Elmer et al. 1987; George 1987; George, in press). The results obtained in these studies and in other recent work by Li and coworkers (Li and Lumeng 1984; Waller et al. 1984) indicate that genetic analyses are important in studies related to the understanding of drug-reinforced behavior. To the best of our knowledge, however, there are no reports on genetic factors in nonethanol operant drug self-administration.

Self-administration procedures utilizing the operant paradigm combine several key criteria necessary for an effective animal model of substance abuse. However, the major focus in self-administration studies has been to determine the environmental conditions important in the initiation and persistence of drug-seeking behaviors. These experiments generally have used a limited number of genetically undefined subjects, and experimental conditions are often varied independently across subjects. Individual differences found in these studies have generally been attributed to differences in training and subject history, or often ignored. It is possible that genetic differences in sensitivity, metabolism, or the reinforcing effects of drugs may have given rise to this variability.

The integration of behavioral genetic and operant methodologies has great potential for increasing our understanding of the contributions and interactions of genetic and environmental factors in determining behavior. The objectives of this integrative approach to pharmacological research would be to identify and study at several levels of scientific analysis, including molecular, cellular, and behavioral, those factors that mediate responses to drugs. The methodology and

principles of operant conditioning and pharmacogenetic analysis can be effectively combined to ascertain the relationship between one operant behavior, such as drug self-administration under fixed-ratio schedules and other operant behaviors, such as food-reinforced behavior, as well as nonoperant behaviors, such as locomotor activity. These comparisons can be used to determine the degree of common genetic control among these factors as well as to establish the extent to which one factor, such as neurosensitivity, covaries with another factor, such as self-administration. These and other issues, such as commonality of self-administration behavior across drugs, can be most effectively addressed by using genetically defined animals within self-administration paradigms.

The purpose of this paper is to (1) summarize findings to date on genetic factors in drug self-administration; (2) present preliminary data from our laboratory showing genetic differences in locomotor stimulant and lethal effects of cocaine; and (3) present preliminary data on genetic differences in cocaine self-administration obtained from a home cage drinking procedure derived from operant self-administration experimental designs.

MATERIALS AND METHODS

Animals

Adult (10 to 14 weeks old at start of testing) LEW (LEW/CRLBR) and F344 (CDF(F-344)CRLBR) male rats were used. All animals were experimentally naive, housed individually (self-administration studies) or in groups of same-sex littermates (locomotor studies) in a temperature-controlled room 26 °C) with a 12-hour light/dark cycle (0700 to 1900 lights on), and given free access to Purina laboratory chow and tapwater prior to initiation of the experiments.

Locomotor Activity Studies

Cocaine, when administered to rats, is known to produce dose-related increases in spontaneous locomotor behavior followed by death at very high doses. In order to examine the role of genetic factors in response to cocaine, we studied the dose-dependent effects of acute cocaine administration in LEW and F344 inbred rats.

Procedure. Six each LEW and F344 rats were individually placed in Digiscan Animal Activity monitors for an acclimation period of 20 minutes, then injected IP with one of several doses of cocaine HCl in

0.9 percent sterile saline expressed as mg/kg freebase. Doses were administered in a volume of 1.0 ml/kg. Rats were immediately returned to the activity monitor, and measures of activity were accumulated electronically and summed every 10 minutes for 60 minutes.

Home Cage Drinking Studies

Apparatus. The animal's plastic home cage served as the experimental chamber. Each cage was equipped with a stainless steel grid top. Standard 100-ml drinking bottles with sipper tubes were used. Two additional cages with bottles but no subjects were used to control for evaporation and spillage.

Procedure. Three each F344 and LEW rats weighing approximately 230 g and 300 g, respectively, at the start of their training were used. Sessions were run 5 days a week between 900 and 1200 hours. The cocaine concentrations (expressed in mg/ml) were prepared using cocaine HCl in tapwater. The stock solutions were prepared, sealed, and stored in a refrigerator. Solutions were periodically checked to control for possible degradation of cocaine. The volumes consumed were measured at the end of each session by weighing the bottles. The animals were weighed daily.

Establishment of water drinking during the testing period. Water bottles were removed from the rats' home cages for 22 hours/day and, to increase further the probability of drinking, the daily feedings of Purina laboratory chow were placed in the cage 60 minutes prior to the beginning of the session.

Induction of cocaine drinking. Presession feedings continued for a series of daily 1-hour sessions. During the first five sessions, 0 mg/ml cocaine (water) was the available liquid, then .05, .1, .2, .4, and .57 mg/ml cocaine for five sessions each. The presession feedings were then gradually reduced, and eventually all food was given to the rats following each session.

Cocaine drinking after the termination of food-induced drinking. After each 1-hour session the rats now had free access to water and their daily food allotment in their home cages. As an initial test of whether cocaine was functioning as a reinforcer, the rats had access to .57 mg/ml cocaine for 10 sessions, followed by 0 mg/ml cocaine (i.e., water) for 10 sessions.

RESULTS

Locomotor Activity Studies

The results are summarized in figure 1. A potency difference was found, with LEW rats being more sensitive and F344 rats less sensitive to the locomotor stimulant effects of cocaine. Conversely, while a difference in lethality response to cocaine was also found, the direction was opposite. All F344 rats but no LEW rats died at a cocaine dose of 60 mg/kg.

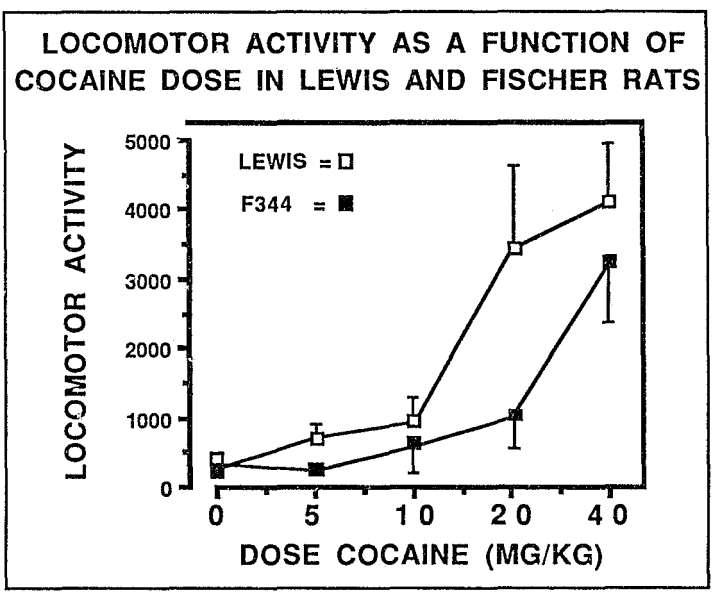

FIGURE 1. *Locomotor activity as a function of cocaine dose in LEW and F344 rats*

Home Cage Drinking Studies

Figure 2 shows that both LEW and F344 rats ingested substantial amounts of cocaine when food induced. Intakes averaged 17.3 and 11.5 mg/kg cocaine and 29.1 and 19.8 μl/g liquid per 1-hour exposure for LEW and F344 rats, respectively. In the absence of food inducement, liquid intake of cocaine solutions was higher in the LEW rats relative to the F344 rats (14.1 vs. 4.8 μl/g and 8.0 vs. 2.7 mg/kg respectively) and exceeded intake of vehicle (8.8 μl/g) in the

LEW animals). Conversely, intake of cocaine solutions was not higher than intake of vehicle (5.4 μl/g) in the F344 rats. This suggests that orally delivered cocaine was serving as a reinforcer only for LEW rats. While not quantified, observations of the LEW rats showed that most intake of cocaine solutions occurred during the initial portion of the test session.

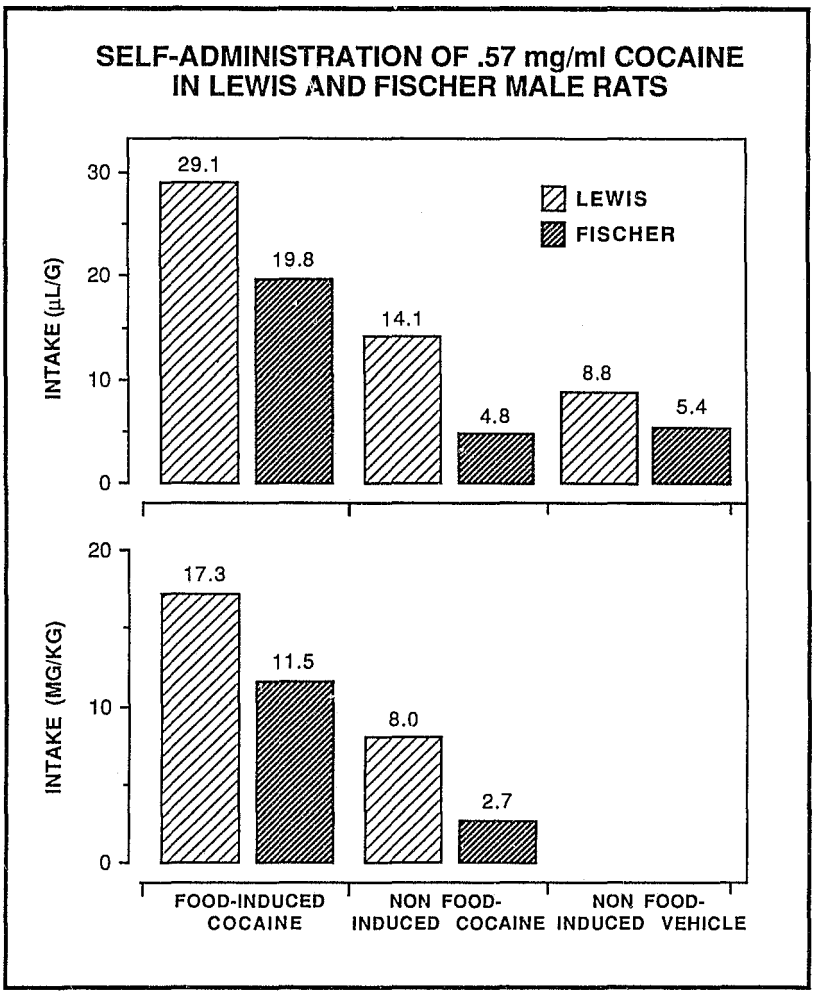

FIGURE 2. *Self-administration of .57 mg/ml cocaine in LEW and F344 male rats*

DISCUSSION

The results of these preliminary studies suggest that significant genetic differences may exist in response to cocaine. In addition, the stimulant, reinforcing, and lethal effects of cocaine may not be highly correlated. Striatal dopaminergic pathways appear to be important in mediating the locomotor-stimulant effects of cocaine (Heikkila et al. 1979), and these pathways have also been shown to be critical for the initiation of reinforcement from cocaine (Ritz et al. 1987). This suggests the possibility that innate differences in striatal dopaminergic pathways may exist between the genotypes used in this study. Although not quantitatively or pathologically determined, death from cocaine in this study was apparently due to effects on cardiovascular tissue. Since sensitivity to the lethal and locomotor stimulant effects does not appear to be correlated, this suggests that the lethal effects of cocaine may not be the result of actions on dopaminergic pathways. Ritz and coworkers (1987) have recently implicated heart muscarinic sites as potent sites for cocaine binding. It may be that the LEW and F344 rats differ with regard to these sites, thus contributing to the difference seen in lethal response to cocaine between these strains.

These are important questions with regard to substance abuse that can be ideally addressed using behavioral genetic methods, particularly genetic correlations. Genetically defined animals can be readily characterized for a given behavioral phenotype and can then be used to test mechanistic hypotheses by studying the correlations between the behavioral phenotype and other measures hypothesized to be related in some manner. A lack of correlation allows for the conclusion that the measures studied are not mechanistically related. A strong positive correlation, especially a perfect rank order correlation between measures, provides strong supportive evidence that the measures are causally related. While pharmacological correlations can be effective in identifying a specific site through which a drug initiates its effects, this site may be necessary but not sufficient for a drug action, and establishing the extent of genetic correlation is a necessary step in completely identifying the biochemical system(s) that mediate a particular drug response.

Behavioral genetics is not only the study of genetic influences upon behavior, but is also, and importantly, the study of environmental influences upon behavior. This latter facet results from the fact that not only can genotype be manipulated as an independent variable, it can also be controlled. When genotype is completely controlled or

held constant, then the resulting variability among subjects is due to environmental factors. When specific environmental variables are being systematically varied, as in most operant studies, the inclusion of complete genetic control through the use of highly inbred subjects can provide a very powerful and repeatable test for the significance of specific environmental factors on behavior.

The results of the present studies are important for several reasons: (1) they illustrate the importance of control for genetic variability in pharmacological research; (2) they provide initial data that can be used in correlational studies dealing with mechanistic hypotheses regarding cocaine's sites of action; and (3) they suggest the existence of populations "at risk" for substance abuse.

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Hepatotoxicity of Cocaine

Louis Shuster, Christine A. Garhart, James Powers, Yona Grunfeld, and Gary Kanel

INTRODUCTION

Fatty liver necrosis in mice that had been fed with cocaine was first described by Paul Ehrlich (1890). This phenomenon was rediscovered independently 87 years later in two laboratories in the United States. Evans et al. (1977) observed that pretreatment with phenobarbital for 4 days decreased the acute toxicity of high doses of cocaine, that is, the incidence of death from convulsions within 3 hours. This pretreatment also produced a marked increase in delayed lethality, that is, deaths from hepatic necrosis during the 7 days after injection of cocaine.

We encountered cocaine-induced hepatotoxicity while studying sensitization to the stimulant effects of cocaine. The surface of livers from mice that had received four or five daily injections of 20 mg/kg cocaine had an unusual pitted or roughened appearance. After other possible causes such as infection had been ruled out, it was found that a single injection of 50 mg/kg cocaine could produce severe fatty necrosis of the liver (Shuster et al. 1977).

THE NATURE OF THE DAMAGE

Histologic examination of cocaine-damaged livers revealed hemorrhagic fatty necrosis that was mainly midzonal and perivenular, with sparing of periportal regions (Shuster et al. 1977; Kanel et al. 1979). This pattern is different from the necrosis produced by carbon tetrachloride and acetaminophen, which is mainly centrilobular. Pretreatment with phenobarbital did cause extension of cocaine-induced damage to periportal areas, in agreement with the findings of Evans and Harbison (1978) and Jordan and Franklin (1978).

Liver damage from cocaine produced a massive release of transaminases into the circulation. Peak levels of serum glutamate-oxaloacetate transaminase (SGOT) were attained at about 16 hours after the injection of cocaine. There was indirect evidence for damage to the smooth endoplasmic reticulum in the form of prolonged sleeptime after the administration of short-acting barbiturates (Shuster et al. 1977). Other workers have reported a diminution of the rate of antipyrine metabolism *in vivo* (Kloss et al. 1983), correlated with a loss of cytochrome P450 (Rauckman 1982a). In addition, electron microscopy by Gottfried et al. (1986) has revealed damage to mitochrondria and peroxisomes.

The dose-response curve for elevation of SGOT showed a threshold of about 40 mg/kg (figure 1). The large standard errors reflect marked individual variability, such as was also encountered when separate mice were tested at various times after cocaine (figure 2). This variability is believed to result from the large number of factors that can affect the hepatotoxicity of cocaine.

STRUCTURAL REQUIREMENTS FOR DAMAGE

Even minor changes in the structure of the cocaine molecule can abolish hepatotoxicity (table 1). The products of deesterification, i.e., benzoyl ecgonine, ecgonine methyl ester, and ecgonine were all ineffective at a dose of 100 mg/kg (Thompson et al. 1979). Removal of the carboxymethyl ester residue at position 2, as in tropacocaine, or even a change in its configuration from axial to equatorial, as in pseudococaine, also abolished hepatotoxic activity (Thompson et al. 1979).

The phenyltropane analog of cocaine, in which a benzene ring is attached directly to the tropane ring (Clarke et al. 1973), was inactive. However, when there was a fluorine on the benzene ring and an isopropyl ester at position 2, the resulting compound was very toxic, as determined by SGOT levels (Thompson et al. 1979). Other tropane analogs such as tropine, atropine, scopolamine, and tropine-N-oxide were inactive (Thompson et al. 1979; Freeman and Harbison 1981b).

THE ROLE OF COCAINE METABOLISM

Metabolites that resulted from oxidation of the N-methyl position by the cytochrome P450 system, that is, norcocaine and N-hydroxynorcocaine, were more potent hepatotoxins than cocaine (Thompson et al.

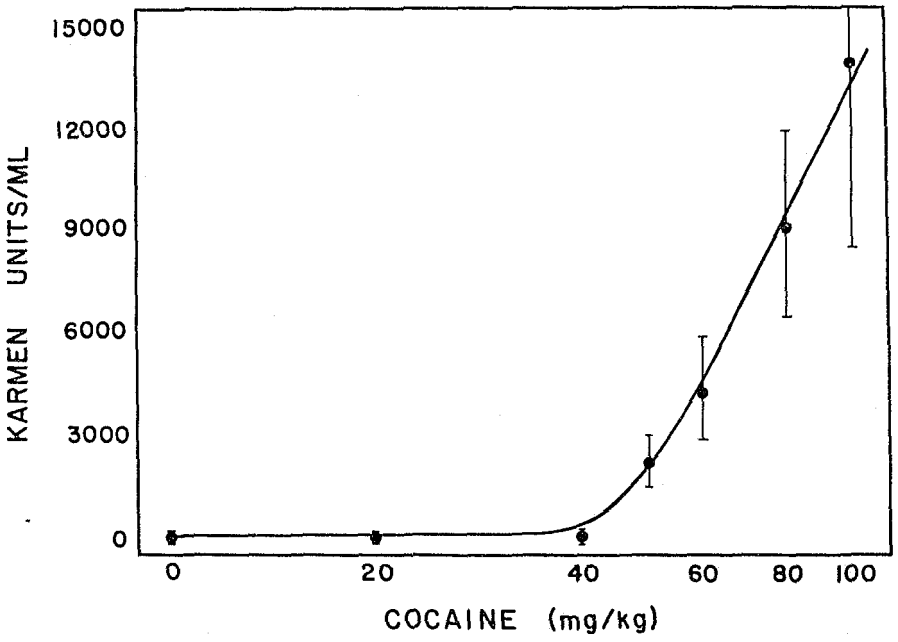


FIGURE 1. *Response of SGOT to different doses of cocaine HCl*

NOTE: SGOT was determined according to Shuster et al. (1978). Blood samples were taken at 24 hours after injection. Each point represents the mean ± SEM for a different group of 10 mice.

SOURCE: Shuster et al. 1977, Copyright 1977, Pergamon Press.

1979). These observations, together with others, suggest that, in order to produce liver damage, cocaine may have to be activated by oxidative metabolism, as is the case with carbon tetrachloride and acetaminophen.

Evans and Harbison (1978) had used phenobarbital, an inducer of the cytochrome P450 system, in order to make mice susceptible to liver damage from cocaine. Such induction did not appear to be necessary in our experiments. However, it turned out that our mice were exposed to a different inducer, that is, softwood bedding (Ferguson

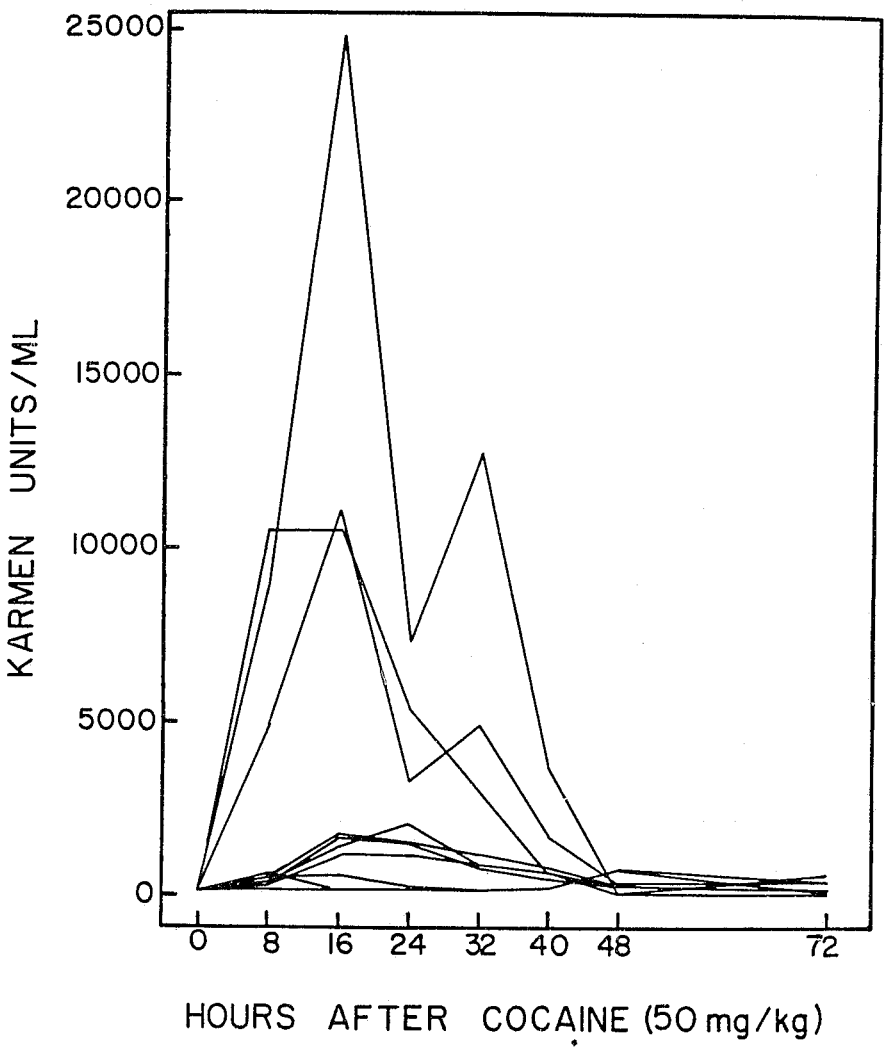
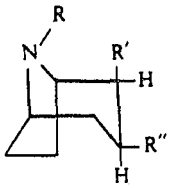


FIGURE 2. *Time course of changes in SGOT after the injection of 50 mg/kg cocaine HCl*

NOTE: Each curve represents an individual mouse.

SOURCE: Shuster et al. 1977, Copyright 1977, Pergamon Press.

TABLE 1. *Structure of cocaine, some metabolites, and related compounds*



Compound	R	R'	R''
Cocaine	CH_3	$COOCH_3$	$OOCC_6H_5$
Norcocaine	H	$COOCH_3$	$OOCC_6H_5$
Benzoylecgonine	CH_3	COOH	$OOCC_6H_5$
Ecgonine methyl ester	CH_3	CH_3	$COOCH_3OH$
Ecgonine	CH_3	COOH	OH
N-hydroxynorcocaine	OH	$COOCH_3$	OOC_6H_5
Tropacocaine	CH_3	H	OOC_6H_5
Phenyltropane 2a	CH_3	$COOCH_3$	C_6H_5
Phenyltropane 22	CH_3	COO-i-pr	C_6H_4F

1966). Beta ionone, an essential oil found in cedarwood, markedly increased susceptibility to liver damage from cocaine. Methyl cholanthrene, an inducer of cytochrome P448, was ineffective (Thompson et al. 1979). As reported by Smith et al. (1981), cocaine is also hepatotoxic when injected into mice that have been induced by pretreatment with ethanol. Liver damage from cocaine could be obtained in the absence of any pretreatment if the cocaine was administered chronically, that is, in the form of two or three daily injections (Freeman and Harbison 1981a; Thompson et al. 1984). This finding raises the possibility that cocaine itself might act as an inducer of the mixed-function oxidase system of the smooth endoplasmic reticulum. Puma and Ramos-Aliaga (1981) have presented evidence that chronic administration of cocaine increases the amount of microsomal protein and cocaine N-demethylase in rat livers.

Inhibitors of the cytochrome P450 system, such as SKF525A, iproniazid, and chloramphenicol, prevented liver damage when injected before cocaine, presumably because they prevent the formation of toxic metabolites (Thompson et al. 1979).

The oxidation of cocaine by the cytochrome P450 system is believed to account for only 10 or 15 percent of overall cocaine metabolism. The remainder is rapidly hydrolyzed by plasma and tissue esterases (Stewart et al. 1978). It is therefore not surprising that the hepatotoxicity of cocaine is markedly potentiated by pretreatment with esterase inhibitors such as diazinon (Thompson et al. 1979) and tri-o-tolyl phosphate (Freeman and Harbison 1981b) even when the cytochrome P450 system has not been induced.

The postulated pathway for the metabolic activation of cocaine is depicted in figure 3. There is good evidence for the *in vitro* formation of norcocaine (Stewart et al. 1978; Evans and Harbison 1978) and N-hydroxynorcocaine (Shuster et al. 1983). These metabolites can also be detected *in vivo,* especially in mice that have been pretreated with an esterase inhibitor (Shuster et al. 1983; Evans 1983; Benuck et al., in press). Our results suggest that the formation of N-hydroxynorcocaine is carried out primarily by the cytochrome P450 system (Shuster et al. 1983). Others claim that the flavin-linked monooxygenase system is responsible for this step (Kloss et al. 1982).

The cytochrome P450-catalyzed formation of norcocaine nitroxide is detectable *in vitro* by electron spin resonance (ESR) spectroscopy (Evans and Johnson 1981; Evans 1982; Rauckman et al. 1982b). This conversion seems to be a necessary step for hepatotoxicity, because inhibitors of the cytochrome P450 system prevent liver damage from N-hydroxynorcocaine (Thompson et al. 1979).

THE ULTIMATE HEPATOTOXIN

Norcocaine nitroxide, being a free radical, was initially believed to be the metabolite that actually damaged hepatocytes, either directly, by reacting with membranes, or indirectly, by depleting glutathione. The loss of glutathione could then permit the buildup of toxic peroxides that are formed in the course of normal metabolism (Evans and Johnson 1981; Rauckman et al. 1982a).

However, it soon became apparent that norcocaine nitroxide is a stable free radical resembling substituted piperidine nitroxides that are used as spin labels. Norcocaine nitroxide is stable in solution and does not react spontaneously with either glutathione or proteins (Rauckman et al. 1982b).

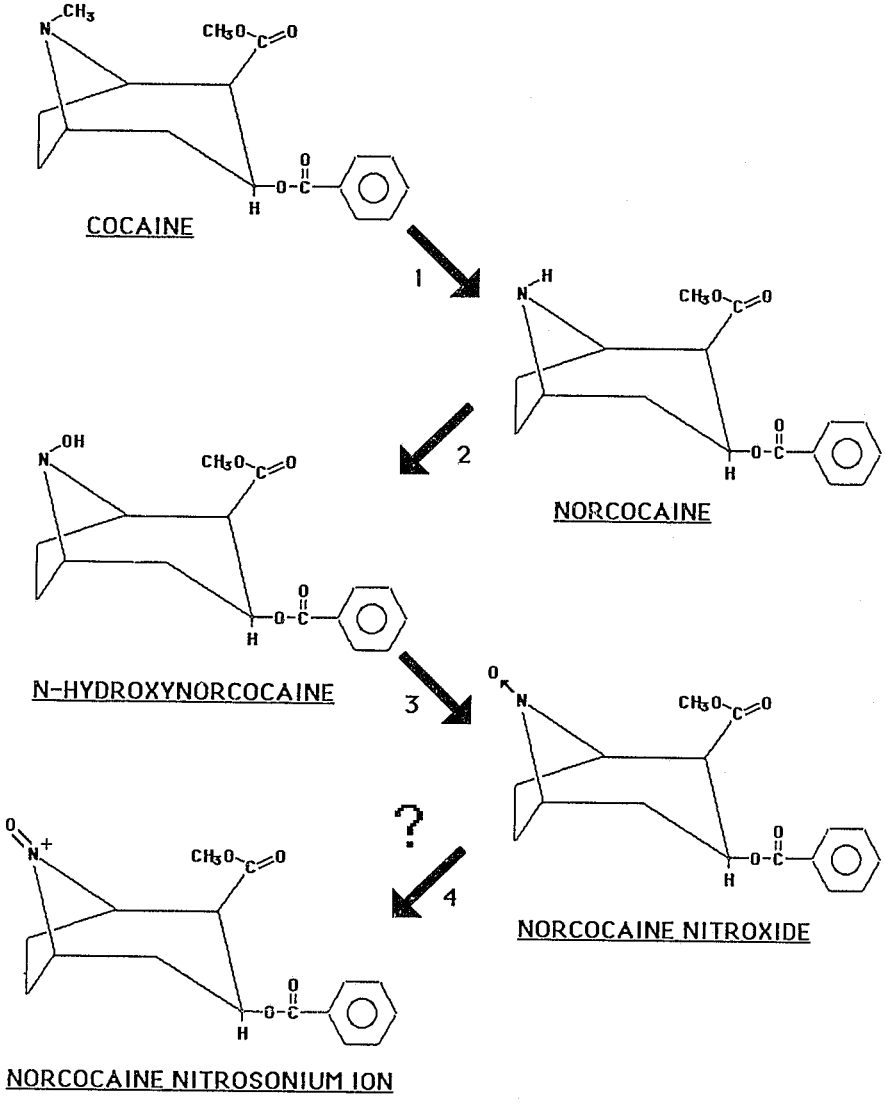


FIGURE 3. *Postulated pathway for the metabolic activation of cocaine to a hepatotoxic free radical*

Rauckman et al. (1982a) and Kloss et al. (1984) have suggested that enzymatically catalyzed cycling between N-hydroxynorcocaine and norcocaine nitroxide may lead to liver damage by depleting NADPH and eventually reduced the amount of glutathione. However, 2,2,6,6-tetramethylpiperidinoxyl (TEMPO) undergoes such cycling (Rosen and Rauckman 1977) (figure 4), and this compound is not hepatotoxic when injected in a dose of 100 mg/kg (Shuster and Powers, unpublished).

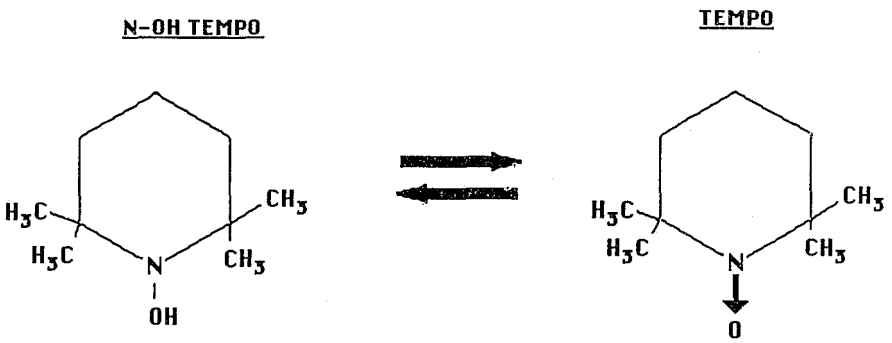


FIGURE 4. *The reversible reduction of 2,2,6,6-tetramethylpiperidinoxyl (TEMPO) to 1-hydroxy-2,2,6,6-tetramethylpiperidine (N-OH-TEMPO)*

NOTE: According to Rosen and Rauckman (1977), the reduction is carried out by cytochrome P450 and NADPH, while the oxidation is catalyzed by a flavin monooxygenase.

We have proposed a different mechanism (Charkoudian and Shuster 1985). The one-electron oxidation of nitroxides such as TEMPO gives rise to extremely reactive nitrosonium ions (Golubev et al. 1965). The nitrosonium ion of norcocaine has been generated by chemical and electrochemical oxidation of norcocaine nitroxide. Such ions react readily with glutathione, and can produce hemolysis of red blood cells *in vitro* (figure 5). Biochemical formation of nitrosonium ions could be catalyzed by cytochrome P450 acting as a peroxidase together with either hydrogen peroxide or a lipid hydroperoxide (Blake and Coon 1981). Semmelhack et al. (1983) and Semmelhack and Schmid (1983) have reported that electrochemically generated nitrosonium ions are very effective oxidizing agents, and can convert alcohols and amines to aldehydes and ketones in high yield. The

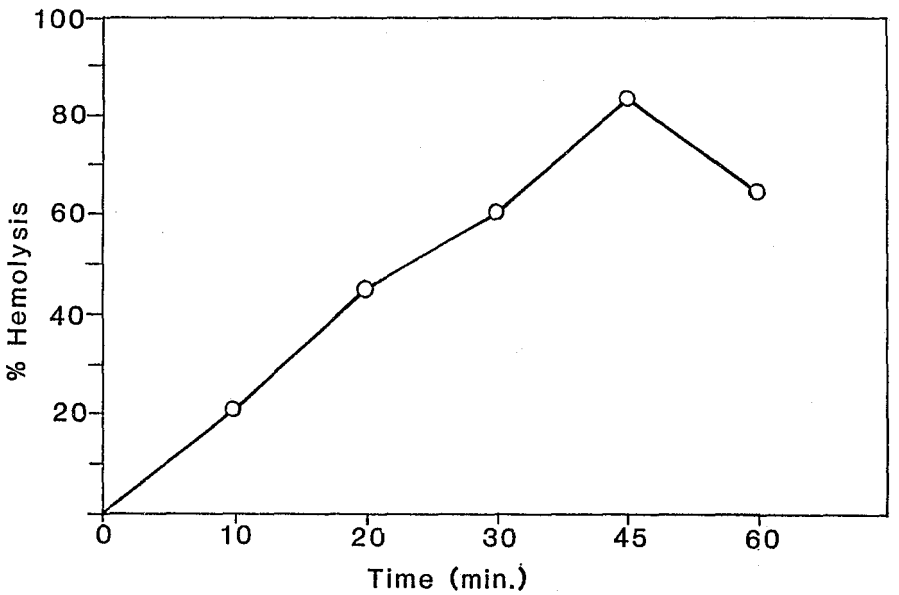


FIGURE 5. *Hemolysis of mouse erythrocytes by the nitrosonium ion of TEMPOL*

SOURCE: Charkoudian and Shuster 1985, Copyright 1985, Academic Press.

resulting N-hydroxy derivative is reoxidized to the nitrosonium ion so that it acts as a catalyst. We have found that nitrosonium ions readily oxidize aspartate to oxaloacetate in aqueous solution. They also oxidize lipids to produce malondialdehyde.

THE POSSIBLE ROLE OF GLUTATHIONE DEPLETION

The hepatotoxicity of cocaine is potentiated when mice are pretreated with diethyl maleate in order to deplete the liver of glutathione (Evans and Harbison 1978). Sulfhydryl compounds such as cysteine and cysteamine protect against liver damage from cocaine (Evans and Harbison 1978; Thompson et al. 1979). These observations suggest that, as in the case of liver damage from acetaminophen, the depletion of glutathione may be an important contributing factor in cocaine hepatotoxicity.

However, liver damage in acetaminophen-injected animals is associated with a decrease of 80 percent or more in the level of reduced glutathione (Mitchell et al. 1973). The extent of glutathione depletion encountered by us (Thompson et al. 1979) and by others (Evans and Harbison 1978) in the livers of cocaine-injected mice was in the range of 20 to 40 percent. A recent abstract by Boyer et al. (1987) reported a decrease of 50 to 65 percent after 60 mg/kg cocaine. Considering that an overnight fast can deplete glutathione levels in mouse liver by 50 percent without causing any liver damage (Strubelt et al. 1981), it appears unlikely that such depletion is by itself the cause of cocaine-induced hepatotoxicity. Even in the case of acetaminophen, it has been found that pretreatment with cimetidine will prevent the liver damage in the face of a marked drop in glutathione levels (Peterson et al. 1983).

The depletion of glutathione from the livers of cocaine-injected mice may be a secondary effect of a primary lesion. Mitochondrial disruption 2 hours after the injection of cocaine has been described (Gottfried et al. 1986). A primary loss of mitochondrial function, particularly oxidative phosphorylation, may be responsible for the 90 percent depletion of ATP after 2 hours, as well as an 85 percent loss of NADPH at 12 hours (Boyer et al. 1987).

LIPID PEROXIDATION

Whether lipid peroxidation is a cause or an effect of liver damage from cocaine is still unresolved. Cornette et al. (1985) did not observe any marked increase in thiobarbiturate-reactive material in the livers of mice that had been injected with cocaine. According to Kloss et al. (1984), the oxidation of cocaine in the liver can lead to the formation of superoxide radicals, hydrogen peroxide, and lipid peroxides. These active forms of oxygen, rather than any free-radical metabolites of cocaine, may be responsible for damage to liver membranes (Rauckman et al. 1982; Rosen et al. 1982). However, this explanation does not account for the covalent binding of labeled cocaine metabolites to liver proteins. This binding correlated with liver damage, and was not seen in rats. More labeled N-hydroxy-norcocaine than labeled cocaine was bound (Evans 1983).

THE ROLE OF CATECHOLAMINES

Recent observations from our laboratory suggest that catecholamines play an important role in liver damage from cocaine.

(1) Adrenalectomy prevents cocaine-induced hepatotoxicity. The effect of adrenalectomy is reversed by subcutaneous injection of epinephrine in oil (Shuster and Thompson 1985).

(2) Pretreatment with alpha blockers such as prazocin or dibenzyline, or depletion of catecholamines by reserpine, also prevents liver damage from cocaine (figure 6). James et al. (1987) have reported that the alpha-adrenergic antagonists

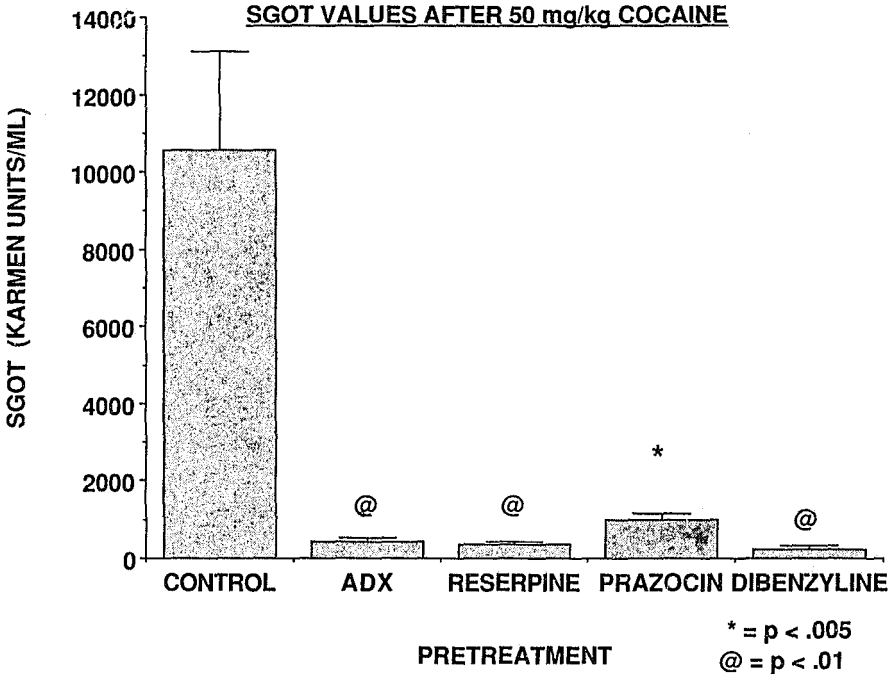


FIGURE 6. *Effect of adrenalectomy and pretreatment with reserpine or alpha blockers on liver damage from cocaine*

NOTE: Sham-operated or adrenalectomized B_6AF_1/J male mice were maintained on 1 percent NaCl in their drinking water for 1 week before being injected with cocaine HCl, 50 mg per kg IP. Pretreatments were: reserpine, 5 mg/kg IP 5 days before cocaine; prazocin, 1 mg/kg 30 minutes before cocaine; dibenzyline, 25 mg/kg 5 hours before cocaine. Each group consisted of four to six mice. Blood samples for SGOT determinations were taken 18 hours after cocaine injection.

phentolamine and yohimbine decreased both liver damage and glutathione depletion by cocaine.

(3) High doses of epinephrine in oil (between 2 and 7 mg/kg) can by themselves produce fatty liver damage and elevation of SGOT levels (figure 7). Other workers have reported that epinephrine can also lower liver glutathione levels (Register and Bartlett 1954; James et al. 1983).

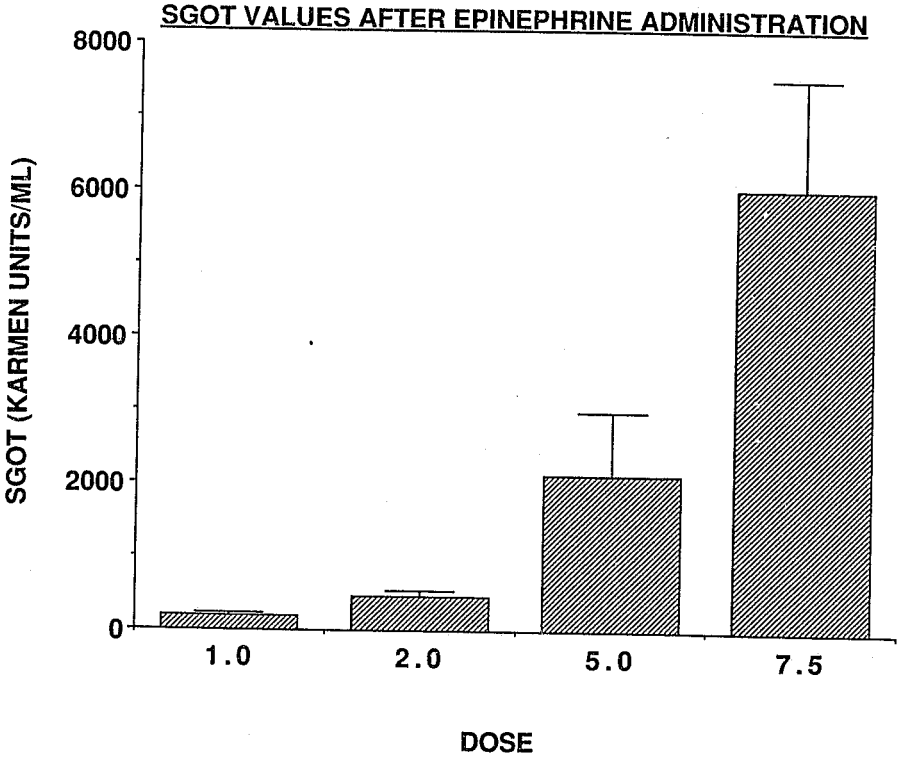


FIGURE 7. *Effect of epinephrine on SGOT levels*

NOTE: Separate groups of four to six male B_6AF_1/J male mice were injected subcutaneously with different doses of L-epinephrine bitartarate in peanut oil. Eighteen hours later, the mice were bled for the determination of SGOT.

(4) The injection of high doses of cocaine increases the levels of circulating epinephrine and norepinephrine twofold to fourfold (figure 8). Similar findings have been made in rats (Chiueh and Kopin 1978).

It is not unreasonable to suggest that catecholamines may, by stimulating hormone-sensitive lipases, cause the release of free fatty acids and increased formation of lipid hydroperoxides. These hydroperoxides, in addition to causing damage directly, could also

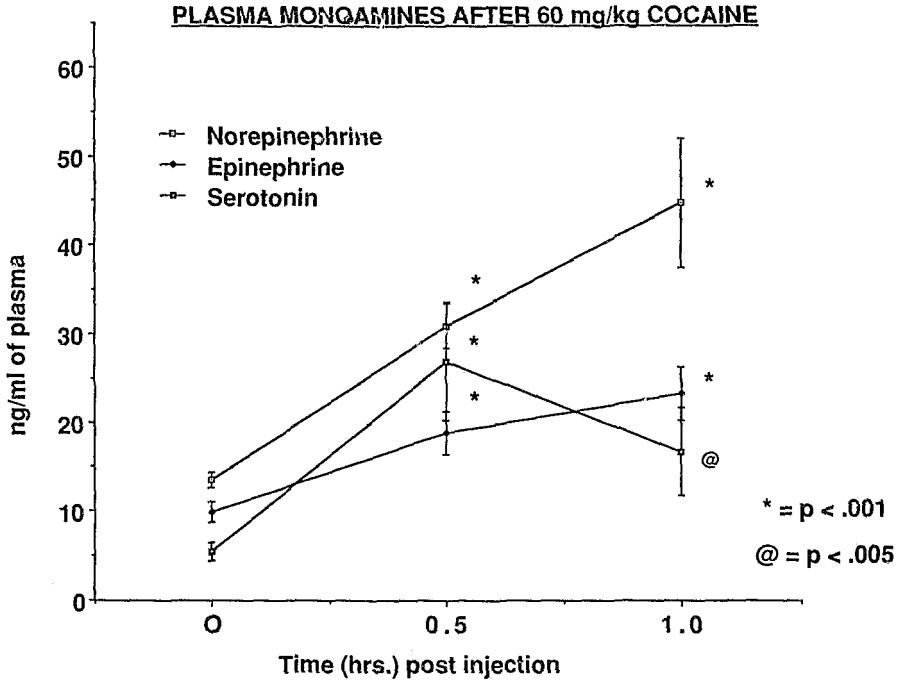


FIGURE 8. *Effect of cocaine on plasma levels of norepinephrine, epinephrine, and serotonin*

NOTE: At each time, a separate group of 10 to 20 male B_6AF_1/J mice were bled from the orbital sinus. Plasma monoamines were determined by HPLC with electrochemical detection.

serve as cofactors for the oxidation of norcocaine nitroxide to the nitrosonium ion.

Those cocaine analogs and metabolites that do not elevate catecholamine should not be hepatotoxic. For example, pseudococaine, which appears to be metabolized like cocaine (Misra and Pontani 1977), even to the formation of a nitroxide (Charkoudian and Shuster, unpublished), is not hepatotoxic. It is also much less effective than cocaine in blocking the reuptake of monoamines (Williams et al. 1977), although it does possess comparable local anesthetic activity (Matthews and Collins 1983).

EARLY CHANGES IN LIVER FUNCTION

The clearance of bromsulfophthalein (BSP) is inhibited by cocaine. An injection of 10 mg/kg, which is not hepatotoxic, will decrease BSP clearance, as demonstrated by elevated plasma levels of the dye, within 1 hour (figure 9). Part of the effect of higher doses of cocaine may be attributed to elevated plasma levels of epinephrine and serotonin, because these substances also interfere with clearance (figure 10).

BSP is extensively conjugated to glutathione before it is excreted into bile. Indocyanine green (ICG) is removed from the liver without prior metabolism. Cocaine did not affect ICG clearance at 1 hour in doses below 60 mg/kg (figure 9). A similar distinction between BSP and ICG clearance was obtained with hepatotoxic doses of carbon tetrachloride. These findings suggest that cocaine may be acting to prevent the conjugation of BSP, perhaps by inhibiting or damaging glutathione transferase.

We have also found that cocaine can alter liver perfusion and bile secretion. When rat livers were subjected to nonrecirculating perfusion with cocaine, there was a dose-related increase in perfusion pressure, an indication that cocaine produces vasoconstriction. Low doses of cocaine increased bile flow, but high doses produced drastic inhibition (figure 11). Similar concentrations of tropacocaine, a nonstimulating analog with local anesthetic activity, and other local anesthetics such as procaine and lidocaine, had little effect on either perfusion pressure or bile flow.

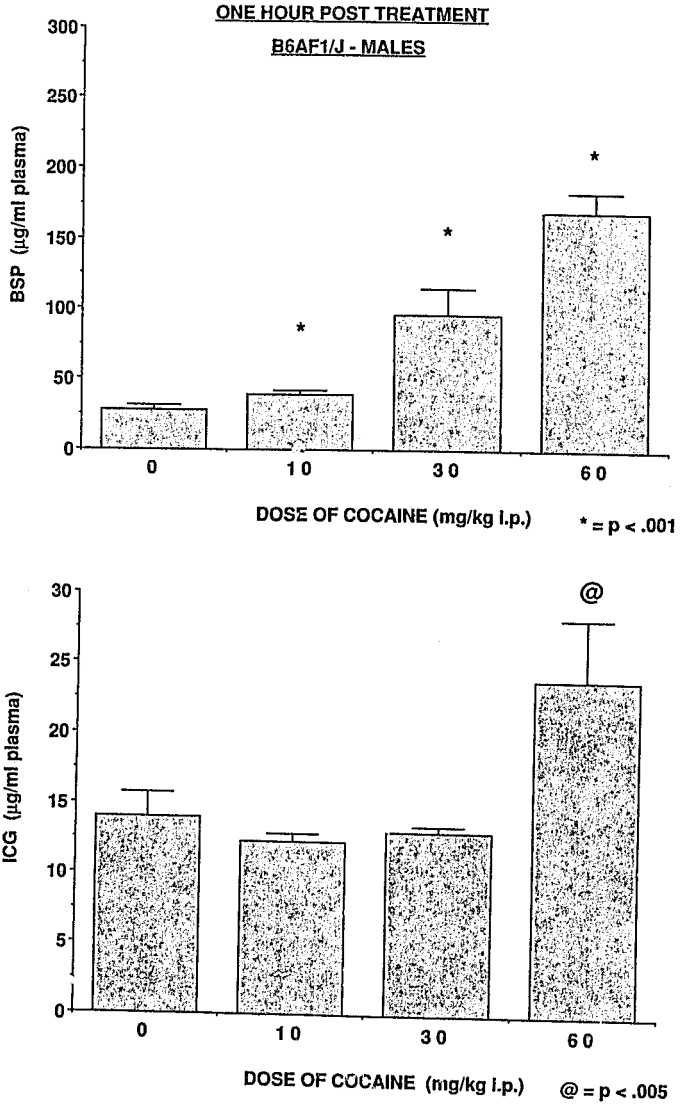


FIGURE 9. *Effect of cocaine on dye uptake*

NOTE: Separate groups of B_6AF_1/J male mice (8 to 10 for BSP, 5 for ICG) were injected with varying doses of cocaine. After 1 hour, they were injected with dye and then bled from the orbital sinus. Plasma dye concentrations were determined according to Hurwitz et al. (1985).

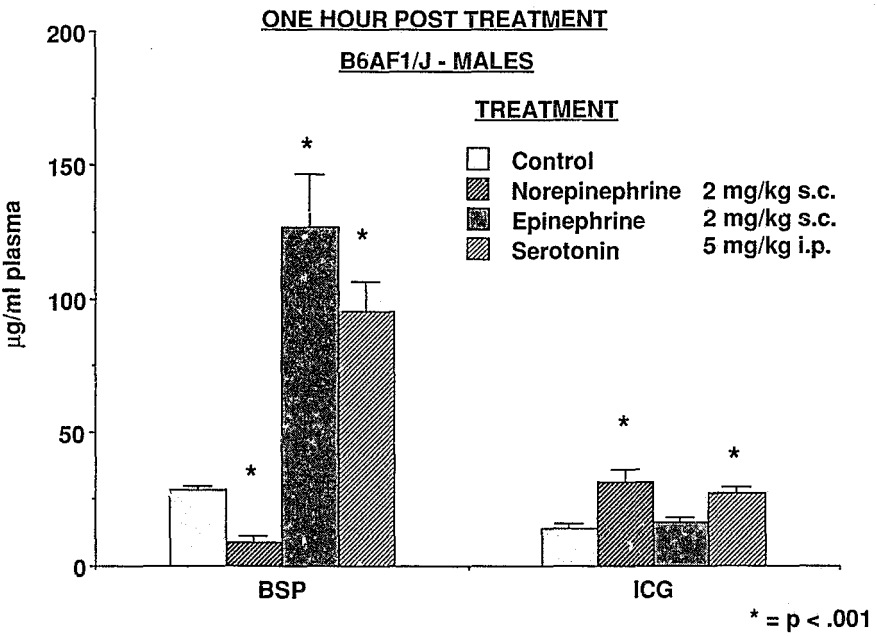


FIGURE 10. *Effect of monoamine injections on dye uptake*

NOTE: The monoamines were injected subcutaneously in peanut oil 1 hour before dye injection. From 6 to 11 male B_6AF_1/J mice per group were used to measure BSP uptake and from 6 to 9 for ICG uptake.

The hepatic vasoconstriction produced by cocaine was not blocked by concentrations of phentolamine that prevented completely the vasoconstriction caused by phenylephrine. This observation suggests that the cocaine is not acting through an adrenergic mechanism.

THE POSSIBLE ROLE OF CALCIUM IONS

Thor et al. (1985) have suggested that oxidative damage to hepatocytes may require the mobilization of calcium ions secondary to a depletion of glutathione. The infusion of norepinephrine into rabbits at a rate of 2 µg/kg/min for 90 minutes produces massive hepatic necrosis and calcium mineralization. These changes are prevented by prazocin (Lee et al. 1986).

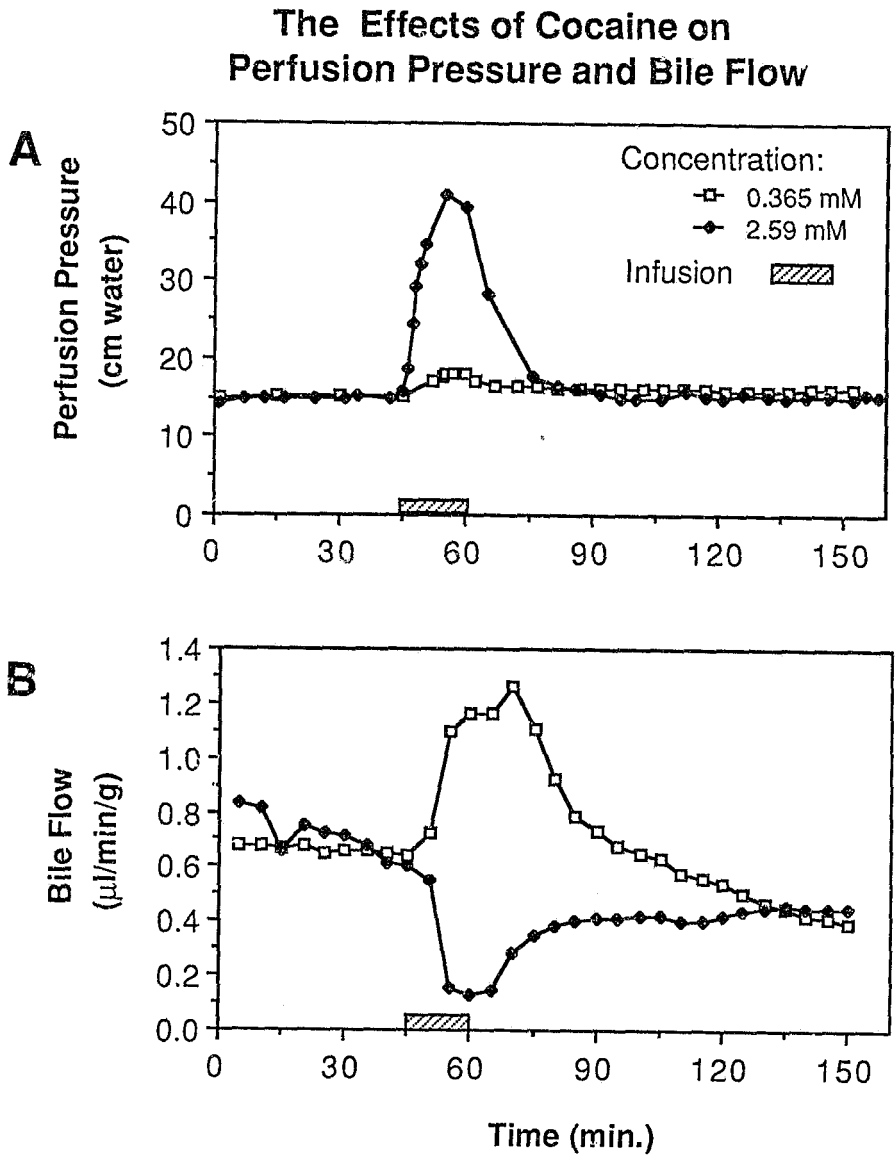


FIGURE 11. *Effects of cocaine on perfused isolated rat liver*

NOTE: A single-pass perfusion was set up according to Anwer and Hegner (1983).

We have added cocaine to isolated rat hepatocytes that had been loaded with the fluorogen Quin 2. In millimolar concentrations, cocaine by itself produced little change in free calcium levels. However, it did inhibit strongly the release of intracellular calcium ions produced by adding epinephrine. There was also inhibition of epinephrine-induced glycogenolysis. Whether these changes are related to the hepatotoxicity of cocaine is unclear, because they can also be produced by somewhat higher concentrations of lidocaine and other local anesthetics. In this case, cocaine is not acting like epinephrine (McConnell et al. 1987).

The direct effects of cocaine on the liver, such as those described here, may involve the specific binding sites for cocaine that have been described by Sershen et al. (1982) and Calligaro and Eldefrawi (1987).

COCAINE-INDUCED HEPATOTOXICITY IN SPECIES OTHER THAN MICE

Species differences are a striking feature of liver damage from cocaine. They may be determined in part by differences in esterase activity and in the inducibility of mixed function oxidases. These variables appear to play a role in the susceptibility of different mouse strains and sexes (Thompson et al. 1984). At least one strain of mice, DBA/2, shows appreciable damage without preinduction of the cytochrome P450 system (Kloss et al. 1982).

Evans (1978) did not find liver damage from cocaine in rats, guinea pigs, or rabbits. We have produced some damage, as indicated by a moderate elevation of SGOT levels, in rats and Syrian hamsters. The increases are much less than those seen in mice, and there does not seem to be any potentiation when the animals are pretreated with phenobarbital. In both species, the response was greater in females than in males (figure 12). Watanabe et al. (1987) have reported that cocaine produces more liver damage in spontaneously hypertensive rats than in Wistar-Kyoto rats.

COCAINE HEPATOTOXICITY IN HUMAN ADDICTS

Until recently, the evidence for cocaine-induced liver damage in humans was limited to a report by Marks and Chapple (1967). These authors described elevated levels of serum transaminases in 80 percent of 89 addicts who were taking both cocaine and heroin.

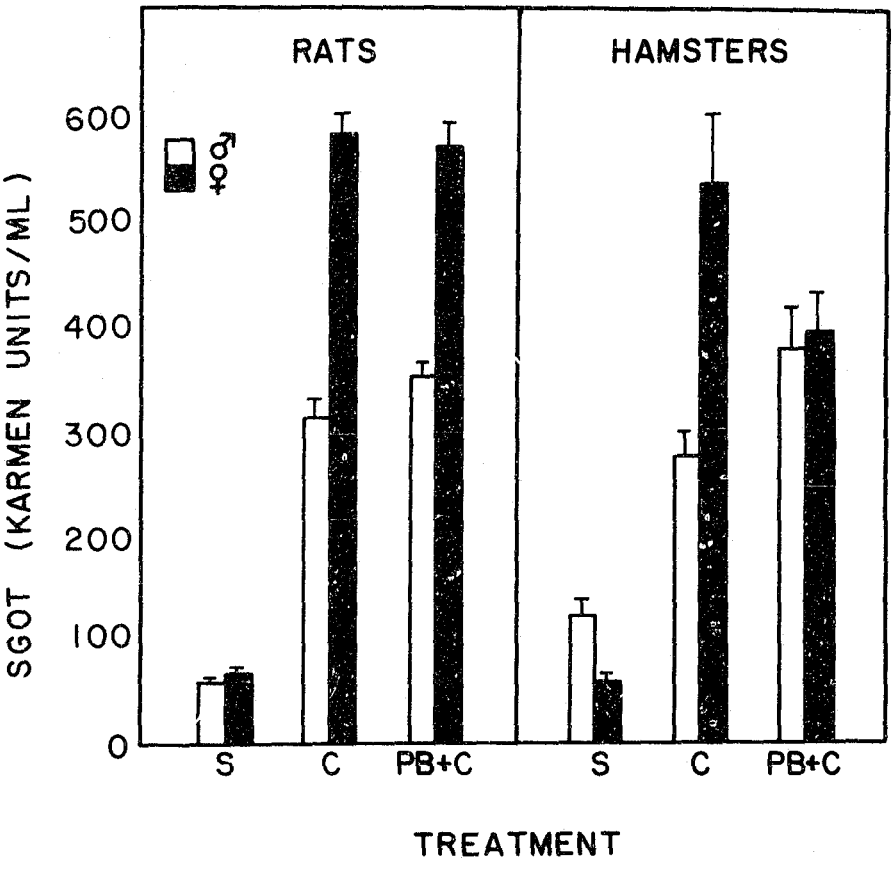


FIGURE 12. *Effect of cocaine on SGOT levels in rats and Syrian hamsters*

NOTE: Pretreatment consisted of four daily injections of either 0.15 M NaCl (S) or phenobarbital, 50 mg/kg (P). On the 5th day, Sprague-Dawley rats were injected with 75 mg/kg cocaine HCl IP (C), and hamsters were injected with 50 mg/kg. Blood was collected 18 hours after cocaine. Each group contained four animals.

Values returned to normal when the drugs were stopped, and went up again upon resumption of the addiction.

This year, Perino et al. (1987) described the case of a 32-year-old man who was abusing a variety of drugs. He was admitted to the hospital exhibiting bizarre behavior after snorting cocaine. His blood tested positive for ethanol, phenobarbital, secobarbital, and methaqualone. Following respiratory arrest, resuscitation, and a downhill course, he died 72 hours after admission. His SGOT level was 620 units/l upon admission, and 10,700 at 28 hours. His serum creatine phosphokinase activity (CPK) upon admission was 7,490 units/l.

At autopsy, there was extensive hepatonecrosis. The necrosis was periportal and midzonal. Centrilobular hepatocytes were intact. Midzonal hepatocytes displayed fatty changes and mitotic regeneration. Perino et al. (1987) emphasized the resemblance of these changes to those observed in cocaine-injected mice.

Five similar cases have now been encountered at the University of Southern California Liver Unit. One was a 20-year-old male who presented with hepatic necrosis, serum glutamate-pyruvate transaminase and SGOT levels in the range of 2,000 to 4,000 units/l, rhabdomyolysis (CPK levels ranging from 14,000 to 28,000 units/l), and renal failure. Death was attributed to submassive hepatic necrosis with pneumonia. The pattern of necrosis was primarily perivenular and midzonal. Periportal hepatocytes showed some fatty changes.

Considering the large number of people who abuse cocaine, it is surprising that such cases are so rare. There is a general tendency to attribute any liver damage in drug addicts to viral infections. Instances of cocaine-induced liver damage in the absence of any viral hepatitis are likely to be uncommon unless one or more of the following precipitating factors are also present:

(1) Pretreatment with inducers of the cytochrome P450 system, such as phenobarbital, phenytoin, or ethanol.

(2) Exposure to esterase inhibitors, such as organophosphate insecticides.

(3) A genetic deficiency of pseudocholinesterase. About 1 person in 2,500 has an atypical level that is 50 percent of normal values. However, this level may still be sufficient for rapid hydrolysis of cocaine (La Du 1971).

(4) Coadministration of cocaine with an opiate such as heroin. Several investigators have shown that narcotic drugs can produce fatty liver infiltration and elevated serum transaminase levels in mice (Thureson-Klein et al. 1978; Needham et al. 1981). We have observed synergistic hepatotoxicity in mice injected with cocaine plus morphine.

Additional information about the requirements for liver damage from cocaine in primates may come from experiments with monkeys. Liver damage has shown up in rhesus monkeys that were injected repeatedly with 32 mg/kg cocaine (Kleven and Woolverton, personal communication, 1987).

CONCLUSION

The oxidative pathway of cocaine metabolism, while relatively minor in comparison to hydrolysis by esterases, gives rise to active metabolites capable of producing severe hepatic necrosis. In addition, cocaine can act directly on the liver to produce vasoconstriction and inhibition of bile secretion. Susceptibility to liver damage from cocaine is influenced by many different factors, so that frank hepatotoxicity is not likely to become a common feature of cocaine addiction in humans. Studies of this phenomenon have contributed to our understanding of cocaine metabolism and its sequelae.

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Lack of Toxic Effects of Cocaine on Dopamine or Serotonin Neurons in the Rat Brain

Lewis S. Seiden and Mark S. Kleven

INTRODUCTION

Due to their euphoric effects, methamphetamine (MA) and related psychomotor stimulants have been abused, at times in epidemic proportions, in various countries including Japan, Great Britain, Sweden, and the United States. Abuse of MA and related drugs often induces short-lasting behavioral episodes that closely resemble paranoid schizophrenia (Snyder 1973). Because of its abuse potential and related behavioral toxicity, there has been considerable interest in assessing possible neurotoxic properties of MA and related compounds.

This chapter will review data indicating that MA and other psychomotor stimulants, as well structurally related compounds, are toxic to dopamine (DA)- and/or serotonin (5-hydroxytryptamine; 5-HT)-containing neurons in brain. This chapter will present data showing that cocaine, a psychomotor stimulant like amphetamine, does not cause neurotoxicity to DA or 5-HT neurons in the central nervous system. The lack of toxic effects of cocaine is similar to that found with methylphenidate (Wagner et al. 1980a). Both drugs are psychomotor stimulants, but neither appears to be toxic to DA or 5-HT cells in the brain. We will also review pharmacological similarities and differences between MA-like compounds and cocaine with regard to neurotoxicity. This difference between cocaine and drugs related to MA that cause neurotoxicity may be due to the intracellular storage pools from which cocaine and MA release monoamines, as well as to the fact that cocaine seems to be a potent DA reuptake blocker. The difference in toxicity between MA and cocaine may thus be related to the differences in the mode of action of the two drugs at the cellular level in spite of similar behavioral effects. Early studies

in rhesus monkeys, rats, cats, and guinea pigs have shown that MA, amphetamine (AMPH), and related phenethylamines are toxic to DA and/or 5-HT neurons (tables 1 and 2). The degree of drug-induced neurotoxicity depends on the duration of drug administration and the dose. Generally, the neurotoxic dose is severalfold (40 to 50 times) higher than the dose required for pharmacological effects such as psychomotor stimulation, anorexia, or stereotypic behaviors, but with some drugs the toxic dose is only slightly (2 to 3 times) higher than the behaviorally effective dose.

TABLE 1. *Neurochemical effects of chronic methamphetamine*

Treatment	Survival	Effect	Reference
3.0-6.5 mg/kg/inj 8 inj/day, 3-6 months	3-6 months	↓striatal DA	Seiden et al. 1975
50 mg/kg/day x 4	2-8 weeks	↓striatal DA ↓^{3}H-DA uptake	Wagner et al. 1980b
15 mg/kg/6 hr x 4	6, 11 days	neuronal degeneration	Lorez 1981
15 mg/kg/6 hr x 5	30 days	↓tryptophan hydroxylase ↓tyrosine hydroxylase	Morgan and Gibb 1980
50 mg/kg/8 hr x 3	2-3 weeks	↑ DA metabolism	Ricaurte et al. 1983
4 mg/day SC inf x 3	2 weeks	↓ striatal DA neuronal degeneration	Ricaurte et al. 1984

KEY: ↑ increased; ↓ decreased.

Early studies with MA have shown that, when this drug is given in relatively high doses for periods of time ranging from 4 to 180 days,

TABLE 2. *Neurochemical effects of chronic 3,4-methylene dioxyamphetamine (MDA) and 3,4-methylene dioxymethamphetamine (MDMA)*

Treatment	Survival	Effect	Reference
MDA			
10-40 mg/kg SC bid x 4 days	2 weeks	↓ 5-HT ^{3}H-5-HT uptake hippocampus neuronal degeneration	Ricaurte et al. 1985
10 mg/kg/6 hr x 5	2 weeks	5-HT, 5-HIAA DA, DOPAC, or HVA tryptophan hydroxylase	Stone et al. 1987
MDMA			
10 mg/kg SC x 1	7 days	↓ 5-HT, 5-HIAA striatum	Schmidt et al. 1986
10-40 mg/kg SC bid x 4 days	2, 8 weeks	↓ 5-HT, 5-HIAA ↓ ^{3}H-5-HT uptake neuronal degeneration	Commins et al. 1987
20 mg/kg SC x 1	7 days	↓ ^{3}H-5-HT uptake	Schmidt 1987
10 mg/kg/6 hr x 5	2 weeks	↓ 5-HT, 5-HIAA ↓ tryptophan hydroxylase	Stone et al. 1987

KEY: ↑ increased; ↓decreased; 5-HIAA 5-hydroxyindoleacetic acid; DOPAC dihydroxyphenylacetic acid; HVA homovanillic acid.

levels of DA and 5-HT are depleted in the brain even several months after the drug is discontinued (table 1). In addition, the DA depletions appear permanent. The number of uptake sites in the high-affinity uptake pump are reduced, suggesting that nerve endings have been lost after MA administration; the affinity constant (K_m) for reuptake did not change, but the maximum number of uptake sites (V_{max}) was lowered. The reduction of high-affinity uptake sites was proportional to the reduction in amine levels. Morphological studies also suggested that neuronal degeneration occurred. The Fink-Heimer method revealed nerve cells in the striatum of rats killed shortly after the MA injection in the process of degeneration (Ricaurte et al. 1982; Ricaurte et al. 1984). The decrease in levels of DA, the loss of reuptake sites, and the morphological evidence indicated that MA had toxic effects on DA neurons. Neurotoxicity has also been reported to occur following repeated administration of the hallucinogenic amphetamines MDA and MDMA (Ricaurte et al. 1985; Commins et al. 1987). That is, long-lasting decreases in 5-HT levels, loss of 5-HT reuptake sites, and signs of neuronal degeneration result from repeated or even single injections of these amphetamine congeners. However, 5-HT neurotoxicity is primarily observed with DA neurons spared under conditions that produce relatively large effects on 5-HT neurons. We conclude from these and other studies (tables 1 and 2) that MA, AMPH, MDA, and MDMA are toxic to DA and/or 5-HT neurons.

In contrast to the prolonged effects reported for amphetaminelike drugs, most previous studies have reported neurochemical effects within 24 hours of the last injection of cocaine (table 3). Taylor and Ho (1976) reported that 45 days of single injections of cocaine (10 mg/kg) caused an increase in particulate tryptophan hydroxylase activity measured 24 hours after the last injection. Since this enzyme is associated with nerve endings, these results suggest that 5-HT metabolism and utilization increased due to the repeated cocaine regimen. However, the same authors later reported that striatal 5-HT levels were slightly decreased (to 86 percent of control), and cytosolic tryptophan hydroxylase activity was reduced as a result of 5 days of single cocaine injections at 10 mg/kg/injection (Taylor and Ho 1977). These effects were also examined 24 hours after the last daily cocaine injection but were not consistent with the increase in 5-HT metabolism observed after 45 days of cocaine administration. With regard to effects of repeated cocaine administration on DA neurons, a similar regimen (10 mg/kg/day for 7 days) produced an increase in striatal tyrosine hydroxylase activity 24 hours following the last injection, with no effect on basal levels of DA or HVA

TABLE 3. *Neurochemical effects of chronic cocaine*

Treatment	Survival	Effect	Reference
Neurochemical			
10 mg/kg/day IP x 45	24 hours	↑ particulate tryptophan hydroxylase activity	Taylor and Ho 1976
10 mg/kg/day IP x 5	24 hours	↓ NE ↓ striatal 5-HT, 5-HIAA ↑ tyrosine hydroxylase activity ↓ tryptophan hydroxylase	Taylor and Ho 1977
160 mg/kg/day SC x 10	10 days	↓ ^{3}H-GABA binding striatum ↑ GABA turnover striatum	Gale 1984
20 mg/kg/day x 10	60 days	tyrosine hydroxylase striatum	Trulson et al. 1986
		↓ tyrosine hydroxylase nucleus accumbens	Trulson et al. 1987
Receptor			
10 mg/kg/day	15-20 minutes	↑ ^{3}H-sulpiride ↓ nucleus accumbens ^{3}H-sulpiride striatum	Goeders and Kuhar 1987
10 mg/kg/day x 7, 14	24 hours	↑ ^{3}H-spiroperidol striatum	Taylor et al. 1979

TABLE 3. (Continued)

Treatment	Survival	Effect	Reference
Receptor (continued)			
20 mg/kg/day x 10	60 days	↑ ^{3}H-spiroperidol striatum	Trulson and Ulissey 1987

KEY: ↑ increased; ↓decreased.

(Taylor and Ho 1977). Roy et al. (1978) also found that acute administration of cocaine no longer decreased DA levels in the striatum after 30 days of twice-daily cocaine injections (15 mg/kg at 8-hour intervals). Although these results suggest a functional change (tolerance) as a consequence of the repeated cocaine dosing, the effect of the repeated administration on levels of DA or 5-HT was not reported. From these previous studies, it is difficult to conclude that cocaine has long-lasting toxic effects on DA and 5-HT. More recent studies have examined longer-lasting effects. Trulson and colleagues (1986) have shown that cocaine treatment in rats twice a day for 10 days at 10 mg/kg/injection causes a reduction in tyrosine hydroxylase immunohistofluorescence staining in striatum 60 days after the last injection of MA. On this basis, they have suggested that cocaine is toxic to the DA system. In addition, they have found that the DA synthesis rate as measured by the accumulation of DOPA was reduced by up to 50 percent in several brain regions of the rat when animals were injected with cocaine on the 10-day regimen (i.e., 10 mg/kg twice a day) (Trulson and Ulissey 1987).

EXPERIMENTAL PROCEDURES AND RESULTS

Experiment 1

Rats were injected twice a day, 12 hours apart, with 10 mg/kg (IP) cocaine hydrochloride and sacrificed 3 or 60 days after the last injection. Brains were analyzed for DA and 5-HT levels in the striatum, hypothalamus, frontal cortex, and hippocampus. There were no changes in the levels of DA or 5-HT either 3 or 60 days after the last injection of cocaine (table 4).

Experiment 2

Cocaine was delivered intravenously with minipumps implanted under the skin and attached to a catheter implanted in the exterior jugular vein. Rats received 100 mg/kg of cocaine per day for 21 days and were sacrificed 2 weeks after removal of the pump. There were no differences in DA or 5-HT values between the cocaine-injected animals and controls (table 5).

Experiment 3

Rats were given 8 IP (100 mg/kg/day) injections 1 hour apart for a period of 10 days. At 24 hours and 2 weeks after the last injection, the animals showed no differences in either DA or 5-HT levels in those regions of the brain examined (figure 1).

TABLE 4. *Effect of repeated daily administration of cocaine (20 mg/kg/day x 10 days) on regional levels of monoamines and metabolites*

Cocaine Group	Cortex	Hippocampus	Hypothalamus	Striatum
5-HT				
3 days	106.1±6.7	84.8±5.3	100.1±13.4	110.2±8.3
60 days	110.6±5.3	97.1±3.3	104.7±6.0	NA
5-HIAA				
3 days	99.2±16.9	86.9±16.3	68.3±13.4	115.1±8.9
60 days	112.2±5.1	102.2±2.7	117.7±5.3	NA
DA				
3 days	ND	ND	108.4±17.3	94.3±6.9
60 days	ND	ND	96.9±6.7	105.3±3.7
DOPAC				
3 days	ND	ND	ND	77.8±9.3
60 days	ND	ND	ND	87.7±7.3

KEY: NA=not available; ND=not determined.

NOTE: Measures represent mean ± SE, percent of control (n=8/group).

The results of these experiments indicate that cocaine does not have long-lasting effects on DA or 5-HT levels in the brain. Since compounds that have been found to be neurotoxic show decreases in transmitter levels (tables 1 and 2), these results suggest that cocaine is not toxic to DA or 5-HT neurons.

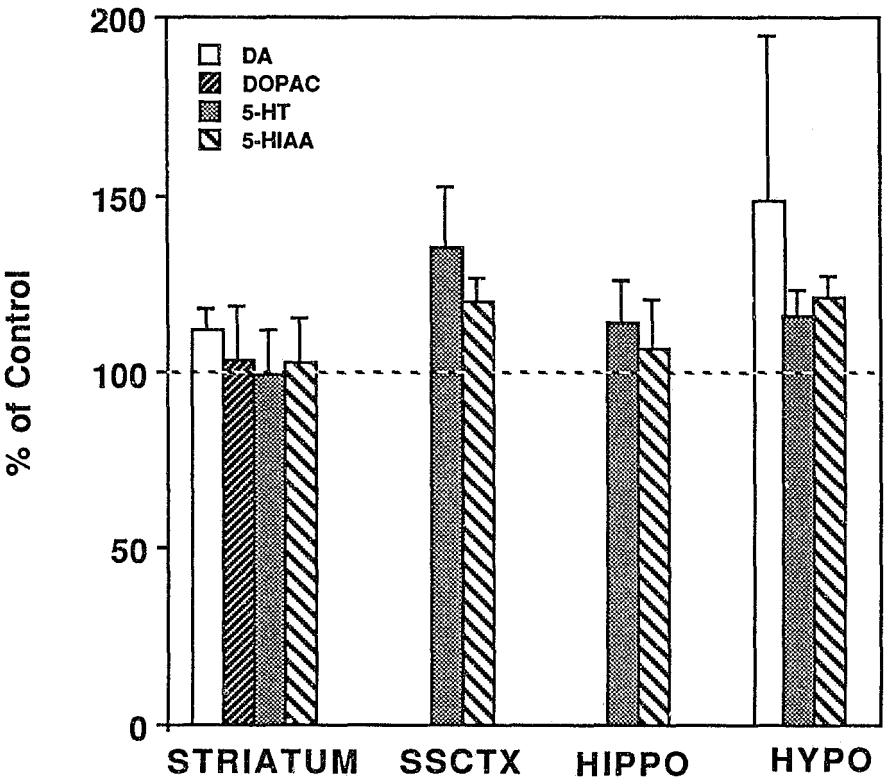

FIGURE 1. *Effect of continuous administration of cocaine (100 mg/kg/day x 21 days) on regional levels of monoamines and metabolites*

NOTE: Rats (n=4) were sacrificed 2 weeks after removal of osmotic minipumps containing cocaine (0.58 g/ml). Values are mean ± SEM of data expressed as a percentage of sham-treated rats (n=5).

DISCUSSION

The results of Trulson and colleagues (Trulson et al. 1986; Trulson et al. 1987; Trulson and Ulissey 1987) are not consistent with the results presented in this paper. We expect that, if levels or activity of the rate-limiting enzyme were decreased, then one would find a corresponding decrease in the amount of transmitter. While our studies are an indirect replication of those of Trulson et al., it would seem that, if the tyrosine hydroxylase in DA-containing areas is reduced, then the amount of DA in those cells should also be reduced. In addition, Trulson and Ulissey (1987) have reported that the synthetic capability of DA cells is reduced to the same extent as tyrosine hydroxylase activity. In this case, one would also predict that the amount of DA should also be reduced, and in the discussion of the paper in 1987, Trulson and Ulissey mention the fact that there was a

TABLE 5. *Effects of repeated daily administration of cocaine (100 mg/kg/day x 10 days) on regional levels of monoamines and metabolites*

Cocaine Group	Cortex	Hippocampus	Hypothalamus	Striatum	Nucleus Accumbens
5-HT					
24 hours	108.7±11.3	88.0±9.4	84.9±7.5	80.7±3.9	97.5±13.3
2 weeks	98.1±3.7	100.0±4.9	92.7±6.9	90.9±10.5	93.5±12.7
5-HIAA					
24 hours	120.4±12.4	116.0±6.5	100.3±7.7	91.7±6.4	139.5±35.3
2 weeks	128.6±9.0	107.4±7.8	99.2±4.4	86.1±4.0	93.9±13.5
DA					
24 hours	ND[b]	ND	101.4±5.2	109.0±6.5	91.7±11.6
2 weeks	ND	ND	91.0±23.1	119.8±4.8	89.1±8.6
DOPAC					
24 hours	ND	ND	ND	117.7±14.2	122.2±15.3
2 weeks	ND	ND	ND	102.1±6.7	82.5±6.4
HVA					
24 hours	ND	ND	ND	135.6±12.9	115.2±20.6
2 weeks	ND	ND	ND	113.4±5.7	95.2±18.4

KEY: ND=not determined.

NOTE: Measures represent mean ± SE, percent of control (n=6/group).

reduction in DA levels. However, our results show no reduction in DA levels following several dosing regimes.

The similarities between the phenethylamine psychomotor stimulants and cocaine in terms of their effects on behavior and their abuse liability would allow one to predict that cocaine, MA, and AMPH may have similar profiles with respect to neurotoxic effects. However, the psychomotor stimulant methylphenidate is also similar in its neurobehavioral actions to MA and AMPH, but does not cause neurotoxicity to either dopaminergic or serotonergic cells (Wagner et al. 1980a). The reasons for the differences in neurotoxic effects of such drugs with similar behavioral effects may be due to differences in their biochemical pharmacology.

Due to the similarities in the behavioral pharmacology but differences in neurotoxicity, it becomes relevant to compare and contrast the effects of AMPH, MA, methylphenidate, and cocaine with regard to their neurochemical mode of action. First, both MA and AMPH cause release of DA from a cytoplasmically bound pool, but do not release from the granular pool (Rateri et al. 1979). MDA and MDMA similarly cause release from the cytoplasmically bound pool of 5-HT and are toxic to 5-HT cells. In contrast, methylphenidate and cocaine are not potent releasers of cytoplasmically bound DA or 5-HT, and insofar as these compounds release DA, they appear to do so from the granular bound pool (Scheel-Kruger 1977).

Evidence suggests that DA release from the granular pool is a necessary factor in toxicity to DA neurons. We have observed that alpha-methyl-para-tyrosine (AMT) blocks toxicity engendered by MA (Wagner et al. 1983; Commins and Seiden 1986). It also blocks behavioral effects of amphetamine, and these actions can be attributed to diminishing the cytoplasmic pool, thereby reducing the releasable amine. Reserpine, which depletes granular bound DA, enhances the toxic effects of MA (Wagner et al. 1983) by shifting the equilibrium between the granular pool in favor of the cytoplasmic pool. In contrast to AMPH, methylphenidate's behavioral effects are blocked by reserpine and are not affected by AMT (Scheel-Kruger et al. 1977), providing evidence that methylphenidate causes release from the granular pool. Similar to methylphenidate, the locomotor stimulant effects of cocaine are not blocked by AMT (Wilson and Holbrook 1979), but are blocked by reserpine (Scheel-Kruger et al. 1972; Scheel-Kruger et al. 1977). Thus, there may be two classes of stimulants: those that utilize the granular pool and those that use the cytoplasmic pool of transmitter.

MA blocks reuptake of DA as do both methylphenidate and cocaine (Heikkila 1975; Koe 1976; Randrup and Braestrup 1977; Taylor and Ho 1978; Church et al. 1987). A salient feature of these neurochemical properties of MA, methylphenidate, and cocaine is the fact that the drugs that show neurotoxic effects have strong releasing actions from the cytoplasmically bound pool, and those that seem to be nontoxic release from the granular pool. This may also explain why methylphenidate is not toxic, because ascorbic acid, which can prevent neurotoxicity, is an antioxidant released with DA from the granule and may protect against the oxidation of DA to 6-hydroxydopamine (6-OHDA). Both ascorbic acid (Wagner et al. 1986) and the antioxidant cysteine (Steranka and Rhind 1987) protect cells from MA toxicity.

The importance of release from different storage pools can be viewed in the context of the properties of the drugs that cause neurotoxicity and the way in which this neurotoxicity can be prevented. According to recent theory (Seiden and Vosmer 1984), by causing release of DA from the cytoplasmically bound pool, MA frees DA to become a substrate for oxidation. Among the oxidation products of DA are trihydroxyphenethylamine derivatives (one of which is GABA), which, through a further oxidation step, can be converted to quinones; the quinone can crosslink proteins, thus causing neurotoxicity within the cell. The fact that 6-OHDA has been detected shortly after the administration of high doses of MA supports this theory (Seiden and Vosmer 1984). The granular pool contains equal molar amounts of DA and ascorbic acid; therefore, release of DA from granules will cause the simultaneous release of ascorbic acid, which protects DA from being oxidized to form 6-OHDA. Since cocaine mainly blocks the reuptake of DA and releases weakly, but only from the granular pool (Koe 1976; Heikkila et al. 1975; Bagchi and Reilly 1983), it would not be expected to be toxic to DA cells. Similarly, methylphenidate releases from the granular pools and is not toxic to dopaminergic cells. Cocaine, like methylphenidate and unlike MA, is not toxic to DA neurons. Both data and theory are consistent with this point. However, it should be noted that the lack of long-term DA depletions does not preclude the possibility that other forms of neurotoxicity might result from repeated cocaine exposure.

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Developmental Effects of Cocaine

Diana L. Dow-Edwards

CLINICAL STUDIES

With the increase in cocaine use among the general population in recent years (Adams and Durell 1984), adverse effects of the drug have occurred in all social strata and age groups, including the pregnant population. For example, approximately 300 to 400 babies are born at the Kings County Hospital (Brooklyn) each year from women admitting to cocaine use (Glass, personal communication, 1987). In 1985, the first well-controlled clinical study appeared, describing the increased incidence of abruptio placentae in cocaine users and neurobehavioral abnormalities in their offspring (Chasnoff et al. 1985). These exposed infants appeared jittery and exhibited decreased interactive behavior and poor organizational responses. Chasnoff et al. (1986) soon reported a case of an infant whose mother used 5 g of cocaine just prior to delivery and who presented with multiple focal seizures, cerebral infarction, tachycardia, and hypertension. Cocaine, or the metabolite benzoylecgonine, was present in the urine of this infant for 4 days. A significant decrease in birth weight and head circumference was found to be associated with cocaine use during pregnancy in a sample of 70 exposed infants and an equal number of matched controls (MacGregor et al. 1987). Others have also reported that cocaine exposure is associated with intrauterine growth retardation, increased stillbirth, and skull defects (Bingol et al. 1987). The first report (LeBlanc et al. 1987) on the effects of intrauterine exposure to the alkaloidal form of cocaine, or "crack," states that exposed infants show abnormal tremulousness, irritability, and muscular rigidity, as well as a decrease in gestation length and birth weight. The clinical literature, therefore, points to neurobehavioral and growth effects on the infant following intrauterine exposure to cocaine.

TERATOLOGIC STUDIES

Even prior to the first clinical report on the effects of cocaine abuse during pregnancy, the teratology of the compound had been described by two independent groups. When mice were examined on gestation day 18 (1 day prior to delivery), Mahalik et al. (1980) found that a single exposure to cocaine at 60 mg/kg SC between days G6 and G12 caused soft tissue and skeletal abnormalities, increased resorptions, and altered the sex ratio. However, these single doses had no effect on maternal weight gain or mean fetal weights. Fantel and Macphail (1982), however, found that repeated cocaine doses of 60 mg/kg IP between days 7 and 16 of gestation in the mouse significantly reduced fetal weights, though, in general, the fetuses appeared to be normal. This same dose, when given daily to pregnant rats between days 8 and 12 of gestation, also caused a decrease in maternal and fetal weights, an increase in the number of resorptions, and the presence of edema in 13 fetuses of 9 litters examined. Higher doses (75 mg/kg) were lethal; lower doses (50 mg/kg) increased the resorption rate. Since the animals were anesthetized with ether prior to the daily injection, the results of this study were questionable. Recently, a fairly complete dose-response curve for subcutaneous administration of cocaine during pregnancy has been established for the rat. Between 40 and 90 mg/kg of cocaine produced decreases in maternal food and water consumption and maternal weight gain in a dose-response manner (Church et al. 1988). Fetal weights were, however, only affected at the highest dose. This relative sparing of the fetuses is perhaps due to the increased fetal fatality observed at all doses of the drug, thereby providing a greater share of the nutrients to the survivors. Fetal edema, abruptio placentae, and cephalic hemorrhage were also identified in the fetuses. Together, the animal data indicate that these consequences--also identified in the clinical population--are effects of cocaine exposure per se and are not merely the result of polydrug interactions, which are so difficult to control in humans.

Spear et al. (1987) have now examined the neurobehavioral development of rats exposed to 40 mg/kg cocaine SC between gestational days 8 and 20. The 40 mg/kg dose had no effect on reflex ontogeny. However, the treated pups showed decreased learning and memory in a test using appetitive conditioning measures as well as a decrease in wall climbing and an increase in locomotor activity following mild foot shock. This dose was found to have minimal effects on the physical development of the fetus as a whole, and no effect on number and weight of live offspring or on gestation length.

Apparently, prenatal cocaine exposure at a dose too small to produce obvious malformations nevertheless does induce abnormal behavioral development, which persists at least until the time of weaning.

BRAIN FUNCTIONAL STUDIES

The effects of cocaine on synaptic development of the brain have been investigated in the rat for the last several years by our laboratory. We have found that cocaine has potent and long-lasting effects on the function of several important cerebral pathways (Dow-Edwards et al. 1986; Dow-Edwards et al. 1988). Our approach has been to administer the drug during the early postnatal period of the rat, when the synapses in the forebrain regions are forming. This is a time approximately equivalent to the third trimester *in utero* for humans. This period is characterized by rapid axonal and dendritic expansion, and is highly sensitive to environmental influences (Dobbing 1968), particularly from agents interacting with the synapse, such as cocaine. As discussed by Balster (this volume), cocaine acts to increase activity at dopamine, norepinephrine, and serotonin synapses. These transmitter substances are important in the functioning of the forebrain regions associated with the reinforcing properties of cocaine (Koob and Hubner, this volume), and are undergoing synaptogenesis during the early postnatal period (Levitt and Moore 1979; Hartley and Seeman 1983; Fillion and Bauguen 1984; Moon-Edley and Herkenham 1984; Murrin et al. 1985). Manipulation of these transmitter substances with agonists or antagonists during critical periods of development can result in long-term alterations in the function of these regions and their associated pathways (Loizou 1972; Coyle and Henry 1973; Rosengarten and Friedhoff 1979; Friedhoff and Miller 1983). Since reuptake of the catecholamines and its inhibition (the presumed action of cocaine on the synapse) are occurring during the early postnatal period (Coyle and Axelrod 1971; Coyle and Campochiaro 1976; Deskin et al. 1981), the mechanisms by which cocaine exerts its effect at the synapse may be operating in the neonate in the same manner as in the adult, but perhaps at a lower level. Therefore, as with other agonists and antagonists, exposure to cocaine during this critical period may alter the function of these synapses both immediately and permanently. The results of our experiments support this idea.

In the first study, we administered 50 mg/kg cocaine HCl to half of the pups in a litter and the vehicle (water) to the other half. During the first 3 days of life, the dose was divided into two portions and administered 8 hours apart. On days 4 through 10, the

injections were administered only once a day in the morning. The litters were culled to eight and were weighed frequently. Reflex ontogeny was assessed on a small sample of pups throughout this early period and was found not to be affected (table 1). Growth of the pups before and after weaning (day 21) was also not affected (figure 1). At 60 days of age, there were no statistically significant

TABLE 1. *Reflex ontogeny of control and cocaine-treated rat pups (mean day of appearance of reflex)*

	Control+ (8)	Neonatal Cocaine (8)
Tail Hang	8.75±0.2	7.75±0.5
Bar Grasp	12.9±0.5	12.7±0.5
Creeping	8.9±0.3	8.5±0.5
Walking	13.2±0.5	13.2±0.2
Eye Opening	14.6±0.3	14.5±0.3

*Significant at $p<0.05$ compared to the control.

+Controls include four untreated and four vehicle-treated rat pups.

differences between groups in mean arterial blood pressure, arterial blood gases or pH, hematocrit, or plasma glucose (table 2). However, the female treated rats showed a significantly different pattern from the untreated females in brain functional activity as measured by glucose metabolism (table 3). Brain glucose metabolism was determined using the deoxyglucose method (Sokoloff et al. 1977) as previously described (Vingan et al. 1986). The female rats were all examined during diestrus to minimize the differences in brain metabolism that occur across days of the estrus cycle (Nehlig et al. 1985). Using the fully quantified method, we found that 40 percent of the limbic structures examined were metabolically stimulated in the treated adult females compared with the female controls. The motor and sensory systems were less significantly affected (table 3).

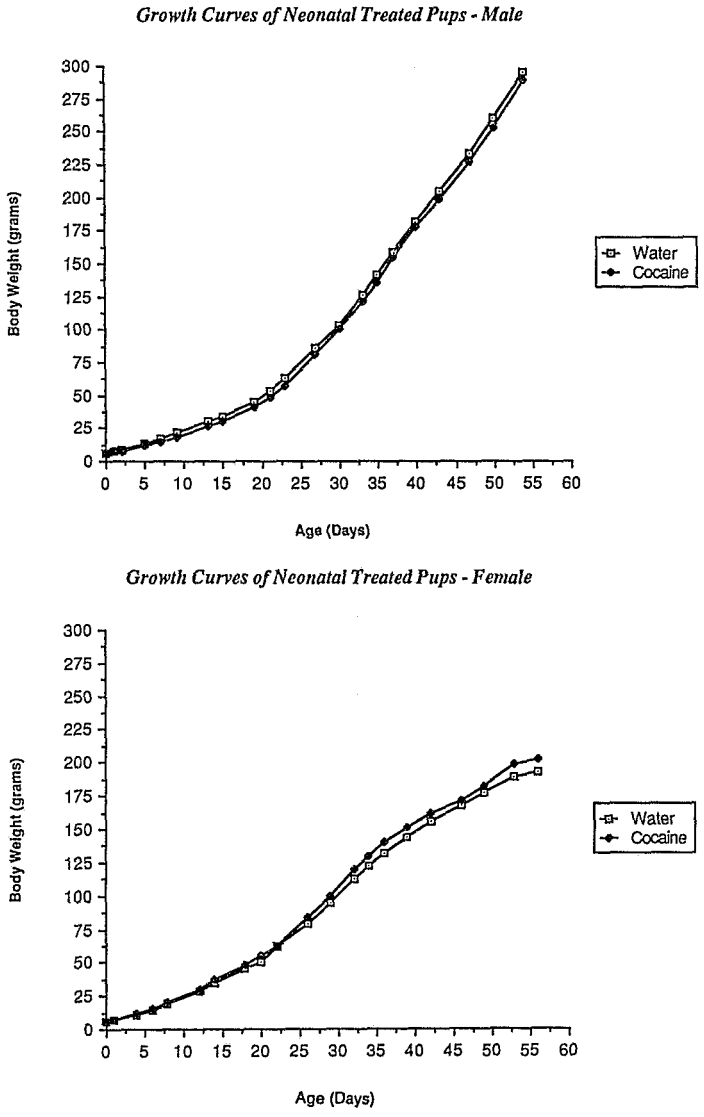

FIGURE 1. *Growth curves of both male (top) and female (bottom) treated and control Sprague-Dawley rats*

NOTE: The treatment consisted of 50 mg/kg cocaine SC between days 1 and 10 of life. Each point represents approximately eight observations. There were no statistically different comparisons between treated and control values.

TABLE 2. *Physiologic parameters of rats treated postnatally with cocaine*

Factor	Female		Male	
	Water n=6	Cocaine n=6	Water n=6	Cocaine n=7
Weight (g)	197±3	204±7	347±16	343±13
Glucose (mg/ml)	1.22±.02	1.22±.07	1.19±.05	1.20±.06
Temperature (°C)	37.9±.18	37.7±.07	37.4±.08	37.4±.13
Pressure (mmHg)	127±3	121±2	120±3	117±2
Hematocrit (%)	48.6±2.1	49.2±1.4	45.7±1.5	47.3±0.9
Gases				
O_2 (mmHg)	89.8±11.5	94.3±6.6	85±5.3	86.3±5.9
CO_2 (mmHg)	24.6±2.1	23.4±2.9	29±2.1	30.9±2.9
pH	7.36±.04	7.38±.05	7.41±.05	7.40±.05
Estrus Cycle	Diestrus	Diestrus		

NOTE: Values are means ± SEM for the number of animals indicated.

Most of the regions examined in the female rats were 10 to 15 percent more active in the treated animals. Every structure found to be significantly altered in this study was also significantly altered by acute cocaine exposure in the adult (Porrino et al. 1988; Porrino and Kornetsky, this volume; London et al. 1986). This is in sharp contrast to the findings with the male rats. Here, neonatal cocaine treatment had little effect on brain glucose metabolism. Table 4 shows a sample of the 43 brain regions evaluated in the male rats. As can be seen, with the exception of the auditory cortex, none of the regions showed a greater than 6 percent difference between the treated and the control groups. Therefore, one might conclude that cocaine administration at 50 mg/kg during the first postnatal week has no significant effect on brain glucose metabolism in male rats, but that it has a large stimulatory effect in females.

Other workers in the field have described similar sex-related differences in response to cocaine. Glick and coworkers (Glick et al. 1983; Glick et al. 1984) reported that cocaine induces a greater amount of turning in females than in males. Behavioral sensitization

TABLE 3. *Brain glucose metabolism in control and cocaine-treated female rats*

	Control (n=6)	Cocaine-Treated (n=6)
Motor Structures		
Motor cx	69±4	79±3
Caudate n	78±4	89±2*
Globus pallidus	45±2	47±1
Thalamus		
Ventral n	67±3	80±2*
Substantia nigra	44±2	48±1
Red n	59±3	68±2*
Pontine nuclei	46±2	51±1*
Pontine reticular formation	48±3	54±3
Vestibular n	96±4	108±5
Cerebellar cx	38±4	41±1
Corpus callosum	36±2	38±2
Limbic Structures		
Mesolimbic forebrain		
Accumbens	74±1	84±5
Bed n stria terminalis	34±1	36±1
Horizontal limb-diagonal	77±3	89±3*
Septum		
Medial	64±2	70±4
Lateral	47±1	48±1
Limbic Cx		
Cingulate cx	86±2	102±4*
Piriform cx	84±5	91±4
Insular cx	112±6	125±6
Hypothalamus		
Medial preoptic n	35±2	36±2
Mammillary body	95±4	108±5
Hippocampus		
CA1	61±3	70±2*
Dentate gyrus	43±3	47±1

TABLE 3. (Continued)

	Control (n=6)	Cocaine-Treated (n=6)
Limbic Structures (Continued)		
Amygdala		
Lateral n	64±2	70±1*
Central n	33±2	37±1
Cortical n	44±1	48±2
Habenula		
Medial n	58±3	67±3
Lateral n	97±2	109±5*
Thalamus		
Mediodorsal n	88±4	106±6*
Ventral tegmental area	51±2	59±1*
Interpeduncular n	83±5	100±5*
Medial raphe n	82±3	99±7*
Central gray	51±3	57±1
Sensory Structures		
Sensory cx		
Head	74±4	83±2
Vibrissa	76±4	83±4
Association (parietal) cx	69±3	76±3
Primary olfactory cx	94±4	106±3*
Occipital cx	77±4	86±4
Lateral geniculate n	69±3	78±2*
Superior colliculus	63±3	73±5
Auditory cx	113±11	130±6
Medial geniculate n	103±8	123±4*
Inferior colliculus	129±11	143±5

*Significant at $p \leq 0.05$ from control by t-test.

NOTE: Measures in μMol/100 g tissue/min; mean ± SEM for the numbers of animals in parentheses.

TABLE 4. *Brain glucose metabolism in control and cocaine-treated male rats*

	Control (6)	Cocaine-Treated (6)
Sensory Structures		
Sensory cx		
Head	85±4	86±2
Vibrissa	86±3	87±3
Association (parietal) cx	80±3	83±3
Primary olfactory cx	117±7	116±5
Occipital cx	96±9	91±5
Lateral geniculate n	81±2	83±3
Superior colliculus	79±5	76±3
Auditory cx	148±7	126±5*
Medial geniculate n	124±3	125±3
Inferior colliculus	153±8	152±1

*Significant value $p \leq 0.05$ from control by t-test.

NOTE: Measures in μMol/l00 g tissue/min; mean ± SEM for the numbers of animals in parentheses.

to cocaine is also more prevalent and longer lasting in females than in males (Glick et al. 1983; Glick and Hinds 1984; Post 1981).

Therefore, it is not surprising that greater alterations in brain function were seen in our experiment in the females exposed to cocaine during synaptogenesis than in the males.

In the next experiment, we wanted to determine whether these functional changes in the female brain were associated with altered behavior in the adult. Therefore, we prepared another 60 females by injecting half with cocaine at 50 mg/kg and the other half with the vehicle (water), following the same injection schedule (postnatal days 1 through 10) and treatments as we had previously. However, this time only the females were studied. Again, at 60 to 65 days of age, the rats (all in the same phase of the estrus cycle) were examined for baseline activity for 15 minutes in a Digiscan Activity Monitor.

This monitor has eight photosensors on each side, as well as a second row of sensors placed 16 cm from the floor of the chamber. It is enclosed in a sound-insulating wood box with two 10-watt lights and has a Par ventilator fan. A window in the center of the top of the box allows the experimenter to observe the animals. Between 10 a.m. and noon, each animal was taken directly from the home cage and placed in the monitor. The behaviors occurring during the 15 1-minute intervals were automatically recorded and grouped into three 5-minute intervals.

As shown in figure 2, neonatal cocaine exposure in female rats increased several types of behavior. Distance traveled per minute (during the second two 5-minute periods), the number of vertical movements per minute (during all time intervals), and the time spent (in seconds per minute) in the center of the monitor (during the first and last 5-minute periods) were all significantly greater in the treated animals than in the controls (by *t*-test analyses). Although all of these data have yet to be analyzed by repeated measures analysis of variance, they do suggest that neonatal cocaine treatment increases general activity levels. However, the increase in time spent in the center of the monitor may be related to the presence of the viewing window in the top of the sound-insulating box; it does not necessarily suggest that the cocaine induced some sort of wall-phobia in these animals.

We have also examined the behavioral responses to amphetamine administration in the neonatally treated female rats, and have found that cocaine appears to increase the sensitivity to amphetamine. Although we have not yet fully analyzed these data, the treated rats appear to show an increase in stereotypic behaviors and a decrease in vertical activity following 1 and 5 mg/kg *d*-amphetamine sulfate IP 30 minutes prior to the behavioral recording. Previous studies have shown that other developmental insults alter responsivity to amphetamine (Silbergeld and Goldberg 1974), and a body of clinical literature indicates that hyperactive children show a paradoxical response to the drug. Our present findings suggest that perinatal exposure to cocaine induces hyperactivity and results in abnormal behavioral responses to amphetamine.

CONCLUSION

Exposure to cocaine during a critical period of development has been shown to produce lasting changes in the function of the brain. If the drug is given during the first 10 days of postnatal life in the rat,

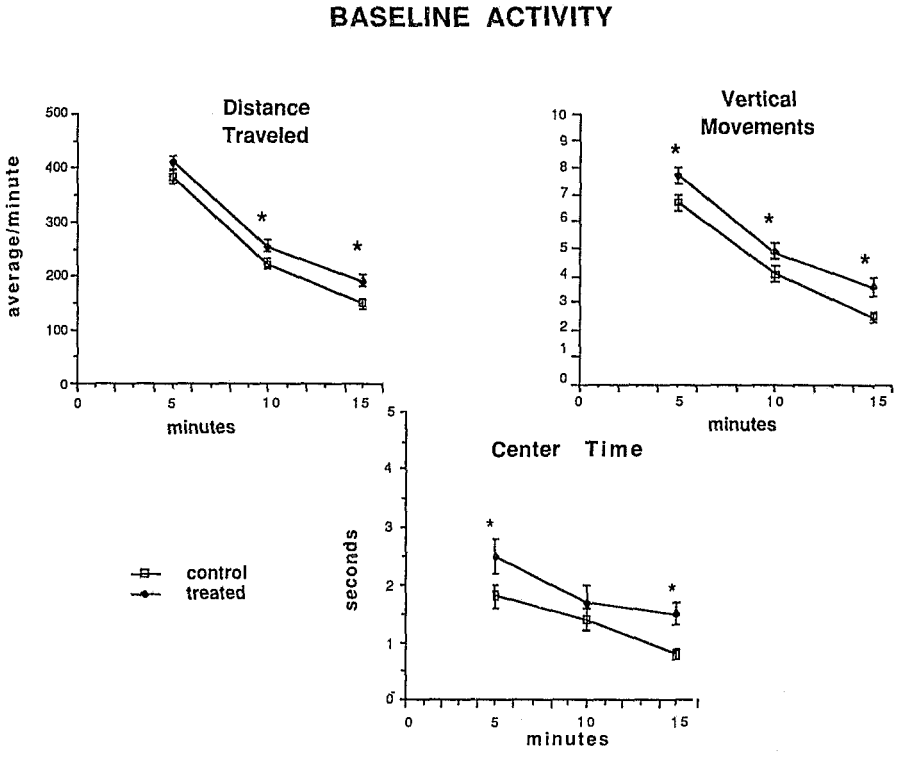

FIGURE 2. *Baseline activity collected with a Digiscan Activity Monitor with vertical sensors 16 cm from the base of the floor*

NOTE: Values ± SD represent data obtained from 60 adult female rats receiving vehicle or 50 mg/kg cocaine between days 1 and 10 of life; data averaged over 5 minutes.

*Significant difference from control by t-test.

▲ is control and O is treated. All animals were in estrus at the time of the behavioral observation.

the animals are hyperactive as adults. Hyperactivity in humans is well known to interfere with performance in school. Therefore, although low to moderate exposure to cocaine during pregnancy does not appear to induce obvious structural abnormalities in the offspring, our data suggest that the drug may place exposed children at risk for neurobehavioral abnormalities that may last into adulthood.

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Cardiovascular Toxicity of Cocaine

R. Douglas Wilkerson

Although cocaine has been utilized for many years as a local anesthetic in the practice of medicine (Ritchie and Greene 1985) and in the research laboratory as a tool to block the neuronal uptake of catecholamines (Trendelenburg 1959), the cardiovascular actions of cocaine itself have not been widely studied. Deaths associated with intravenous cocaine abuse, however, have been reported over the last 10 years, and the circumstances surrounding these deaths were clearly consistent with sudden cardiac death (Lundberg et al. 1977; Di Maio and Garriott 1978; Kossowsky and Lyon 1984; Nanji and Filipenko 1984). Recently, Mittleman and Wetli (1987) restudied autopsy material from 24 patients who had apparently suffered sudden "natural" deaths and found that cocaine was present in the blood of 11 of the 24 patients. Cerebrovascular complications have also been reported after cocaine use, and these reports have recently been the subject of a review by Levine and Welch (1987).

The vast majority of recent reports of cocaine-associated cardiovascular toxicity have implicated cocaine in episodes of myocardial ischemia, which frequently resulted in myocardial infarction. These reports have been the subject of recent reviews by Mathias (1986) and Duke (1986). In addition, Smith and coworkers (1987) recently reviewed the pertinent data from all 38 patients reported to have suffered myocardial ischemic episodes temporally associated with cocaine use. A noteworthy finding was that a significant number of patients with documented evidence of myocardial infarction were subsequently shown to have angiographically normal coronary arteries. This finding of normal coronary arteries subsequent to a documented ischemic episode, temporally related to cocaine use, has led a number of investigators to suggest that cocaine may induce coronary vasospasm (Schachne et al. 1984; Isner et al. 1986; Zimmerman et al. 1987)

and/or promote coronary thrombosis (Zimmerman et al. 1987; Gardezi 1987; Kirlin et al. 1987). Although there is only circumstantial evidence of cocaine-induced coronary vasospasm, cocaine has been shown to cause vasospasm in other vascular beds (Altura et al. 1985).

The cardiovascular actions of cocaine are extremely complex. As a result of its local anesthetic actions, one might expect vasodilation in most vascular beds and a reduced tendency for cardiac arrhythmias. On the other hand, the excitatory effects of cocaine, like other local anesthetics in the central nervous system, might be expected to increase peripheral sympathetic tone (Ritchie and Greene 1985). This central stimulation of sympathetic outflow, combined with cocaine's well-established action to inhibit the neuronal uptake of norepinephrine, would be expected to elicit intense vasoconstriction in most vascular beds, an increase in heart rate and myocardial contractility, and an increased tendency for cardiac arrhythmias. These factors would also lead to an increase in myocardial oxygen consumption. Thus, the two best-understood actions of cocaine (i.e., local anesthesia and inhibition of neuronal uptake of catecholamines) would be expected to produce opposing effects on the heart and circulation. The local anesthetic effect on the heart and blood vessels would result in antiarrhythmic and vasodilatory actions, while local anesthetic actions and effects at adrenergic, dopaminergic, and serotonergic synapses within the central nervous system would result in excitation leading to seizure activity and increased peripheral sympathetic tone. The effects of the norepinephrine released by this increased sympathetic tone to the heart and blood vessels should be potentiated by the action of cocaine to inhibit the major mechanism for terminating the action of norepinephrine, that is, neuronal uptake. Because of this complexity of actions, it is essential that studies designed to clarify the cardiovascular actions of cocaine be performed utilizing a model that preserves all aspects of cocaine action. This can only be accomplished in an intact, conscious animal model, which allows the integrated central nervous system and cardiovascular actions of cocaine to be fully expressed without interference from general anesthesia.

To better understand the contribution of the central nervous system to the cardiovascular actions of cocaine, we compared the effects of intravenous cocaine, 1 mg/kg, on blood pressure in conscious dogs with the effects of the same dose in dogs anesthetized with 32 mg/kg pentobarbital sodium (figure 1). In anesthetized dogs, this dose of cocaine produced a 9.5±3.1 mmHg increase in blood pressure, while in conscious dogs, the same dose of cocaine produced a 45.8±5.2 mmHg

elevation in blood pressure (Wilkerson 1988). Thus, it is clear that depression of the central nervous system significantly reduced the peripheral vascular actions of cocaine which, presumably, were mediated, at least in part, by increased peripheral sympathetic tone resulting from its central stimulant actions.

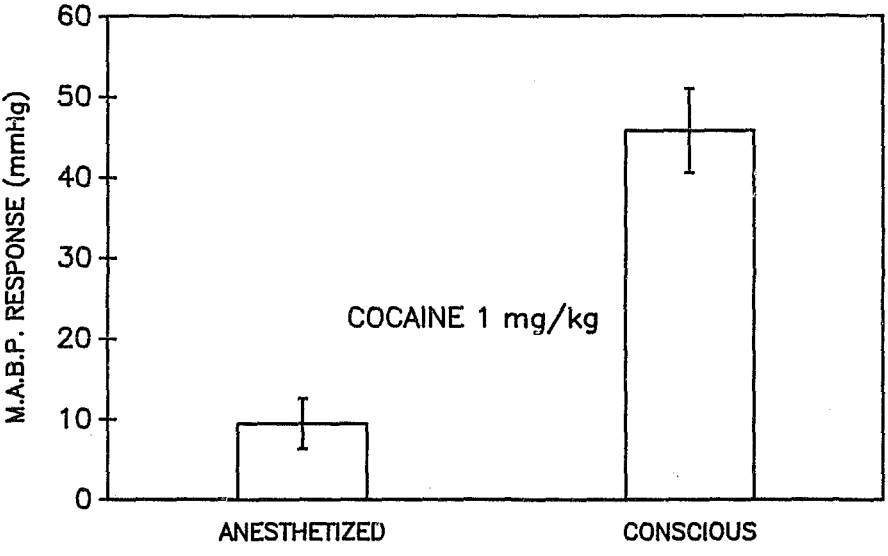

FIGURE 1. *Influence of pentobarbital anesthesia on the blood pressure response elicited by 1 mg/kg of cocaine administered intravenously in dogs*

NOTE: The bar on the left illustrates the blood pressure response to 1 mg/kg of cocaine in a group of 10 dogs anesthetized with pentobarbital sodium (32 mg/kg). The bar on the right illustrates the blood pressure response to the same dose of cocaine in a group of 10 trained conscious dogs. Each bar represents the mean ± standard error for the number of animals indicated.

To confirm the importance of a central nervous system component to the cardiovascular effects of cocaine, we compared the effects of cocaine on blood pressure in conscious dogs with its effects in the same animals, on a different day, after pretreatment with 10 mg/kg of hexamethonium, a ganglionic blocking drug. This dose of hexamethonium did not significantly alter blood pressure, but did significantly increase heart rate. In addition, hexamethonium abolished

the reflex increase in heart rate elicited by intravenous administration of nitroglycerin. It can be seen in figure 2 that this dose of hexamethonium significantly reduced the pressor response elicited by the intravenous administration of 2 mg/kg of cocaine from 70±4.2 to 25.3±3.8 mmHg. The fact that hexamethonium itself at this dose had very little effect on blood pressure suggests that sympathetic

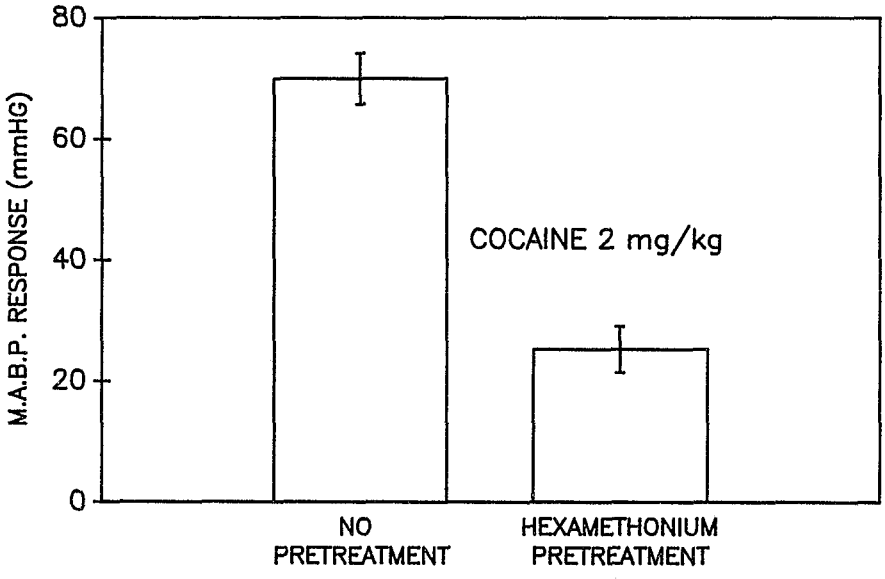

FIGURE 2. *The effect of ganglionic blockade with hexamethonium on the blood pressure response to cocaine (2 mg/kg, IV) in trained, conscious dogs*

NOTE: The left bar depicts the response of six dogs to this dose of cocaine without any pretreatment. The right bar depicts the response to this same dose of cocaine in the same six dogs on a different day, but after pretreatment with 10 mg/kg of hexamethonium. Each bar represents the mean ± standard error of six dogs.

tone to blood vessels in these conscious dogs was quite low (Wilkerson 1988). This observation argues against the possibility that the major action of cocaine to increase blood pressure was merely by blocking neuronal norepinephrine uptake and, thus, potentiating the effect of norepinephrine being released as a result of normal sympathetic tone. These findings of a pivotal role of the central

nervous system in the cardiovascular actions of cocaine are in accord with the findings of Matsuzaki and coworkers (1978), who have demonstrated that EEG changes and behavioral hyperexcitation accompanied the cardiovascular effects of cocaine in subhuman primates.

It would be expected that the action of cocaine to inhibit the neuronal uptake of norepinephrine at peripheral adrenergic synapses should play a major role in the cardiovascular effects of this drug. Indeed, cocaine potentiates and prolongs the effects of exogenously administered norepinephrine (Trendelenburg 1959; Whitby et al. 1960). Despite this well-documented action to potentiate the effects of exogenously administered norepinephrine, however, the effects of cocaine on the response to sympathetic nerve stimulation are still debated. It might seem straightforward that, if cocaine inhibits the neuronal uptake of norepinephrine, then the norepinephrine released from sympathetic nerves should accumulate in adrenergic synapses and thus the effects of sympathetic nerve stimulation should be potentiated. Indeed, some studies have reported that cocaine does increase the effects of sympathetic nerve stimulation (Moore 1966; Langer and Enero 1974). Yet, other investigators have been unable to observe any potentiation of the effects of sympathetic nerve stimulation by cocaine (Koerker and Moran 1971; Matsuda et al. 1979; Levy and Blattberg 1978). Moreover, Koerker and Moran (1971) demonstrated that cocaine pretreatment did not increase the amount of norepinephrine in the venous outflow from the heart during sympathetic nerve stimulation as would be expected if the synaptic concentration of norepinephrine was greatly increased.

This apparent difference in the effects of cocaine on the actions of exogenously administered norepinephrine and on the actions of sympathetic nerve stimulation (i.e., endogenous norepinephrine) is undoubtedly the result of synaptic control mechanisms, by which synaptic norepinephrine effectively controls the rate of its own release by activating presynaptic α_2-adrenergic receptors (Yamaguchi et al. 1977). Inhibition of neuronal catecholamine uptake may momentarily result in an increase in synaptic norepinephrine, but its action on presynaptic α_2-adrenergic receptors would decrease further release of norepinephrine. This control mechanism is "bypassed" when norepinephrine reaches the synapse via a process that does not require its neuronal release (e.g., intravenous injection).

Recently, it has been demonstrated that, even after inhibition of the neuronal uptake of norepinephrine, the amount of norepinephrine in

the synapse is not elevated when sympathetic tone is relatively low (Cousineau et al. 1986). These same workers also demonstrated, however, that although this negative feedback control of norepinephrine release was very effective at low to moderate rates of sympathetic nerve stimulation, at high rates of sympathetic nerve stimulation, this control mechanism was not as effective, and synaptic norepinephrine levels were increased after neuronal uptake inhibition. This suggests that the efficacy of the negative feedback control of norepinephrine release is not sufficient to effectively control synaptic norepinephrine concentration when sympathetic tone is elevated. It is only in this setting that inhibition of the neuronal uptake of norepinephrine leads to its accumulation in the synapse. Thus, when inhibition of norepinephrine neuronal uptake can be matched effectively by inhibition of its neuronal release, synaptic norepinephrine concentration will not increase, but when this is not the case (e.g., when sympathetic tone is elevated), synaptic norepinephrine concentration will be increased by inhibition of neuronal uptake.

In addition to the control mechanism described above, Cohen et al. (1984) have described a second presynaptic mechanism by which the rate of norepinephrine release may be controlled. These workers have shown that acetylcholine inhibits the release of norepinephrine from adrenergic neurons and that this action is blocked by muscarinic antagonists such as atropine. The involvement of these and perhaps other synaptic processes acting to control the rate of norepinephrine release may explain the divergent results of some workers who have attempted to study the effects of cocaine on the cardiovascular actions of sympathetic nerve stimulation. Undoubtedly, some of the confusion also results from the fact that these studies employed relatively large doses of cocaine (5 mg/kg) and that they were performed in anesthetized animals.

As shown in figures 1 and 2, the cardiovascular actions of cocaine clearly have an important component that involves the central nervous system, which results in an increase in sympathetic outflow to the periphery. Thus, the cardiovascular actions of cocaine are not solely dependent upon inhibition of neuronal uptake of norepinephrine at peripheral adrenergic synapses to provide increased synaptic concentrations of norepinephrine. In addition to blocking uptake, cocaine apparently increases sympathetic tone, as evidenced by the action of hexamethonium to inhibit its pressor response. Chieuh and Kopin (1978) have also demonstrated that cocaine exerts an action in the central nervous system to cause the release of norepinephrine and epinephrine from the sympathoadrenal axis in conscious rats.

Furthermore, cocaine appears to cause the release of norepinephrine from isolated cardiac atrial tissue (Trendelenburg 1968). Thus, the adrenergic actions of cocaine cannot be thought of as simply the result of peripheral inhibition of neuronal catecholamine uptake. The central stimulant component of its cardiovascular effects is probably what separates cocaine from other drugs, such as the tricyclic antidepressants, which also very effectively inhibit catecholamine uptake, but whose cardiovascular actions are not as prominent as those of cocaine.

We have recently demonstrated the importance of these synaptic control mechanisms in modulating the cardiovascular actions of cocaine (Wilkerson 1987). Yohimbine, a selective α_2-adrenergic antagonist (Goldberg and Robertson 1983), which is widely abused for its alleged aphrodisiac properties (Linden et al. 1987), was utilized to assess the influence of presynaptic adrenergic control of norepinephrine release on the actions of cocaine. This drug was employed alone and in combination with atropine which, as described above, inhibits cholinergic mechanisms that have also been shown to modulate norepinephrine release.

Yohimbine, 0.25 mg/kg, increased the effects of cardiac sympathetic nerve stimulation on heart rate at low stimulation frequencies, but failed to alter the frequency-response curve at higher frequencies (figure 3). This finding is in accord with Cousineau and coworkers (1986), who demonstrated that the presynaptic α_2-adrenergic mechanism for controlling norepinephrine release is most effective at low stimulation frequencies. In contrast, cocaine, 1 mg/kg, increased the effects of sympathetic nerve stimulation on heart rate at higher frequencies, but not at low frequencies (figure 4). This is not surprising, since, at higher stimulation frequencies, where feedback control of norepinephrine release is less effective, inhibition of neuronal uptake of norepinephrine may not be countered by a corresponding decrease in its neuronal release. This would allow the synaptic concentration of norepinephrine to rise and the effects of sympathetic nerve stimulation to be potentiated by cocaine.

Figure 5 illustrates the effects of inhibition of either or both of the previously described mechanisms controlling neuronal release of norepinephrine on the blood pressure response to intravenous cocaine, 1 mg/kg, in anesthetized dogs. The effect of cocaine alone at this

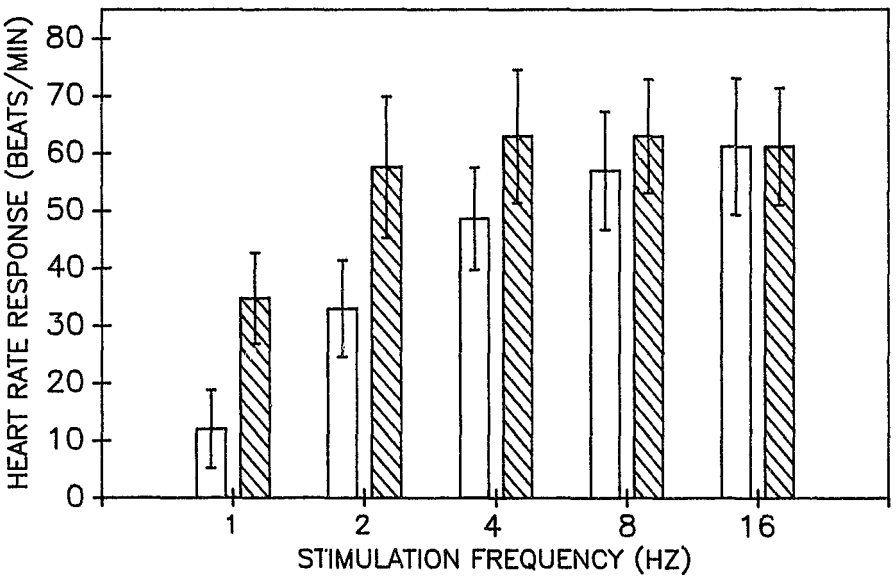

FIGURE 3. *Influence of pretreatment with the α_2-adrenergic antagonist yohimbine on the heart rate response to cardiac sympathetic nerve stimulation in anesthetized dogs*

NOTE: The left ansa subclavia was electrically stimulated at the frequencies shown on the abcissa prior to (open bars) and after (hatched bars) treatment with yohimbine (0.25 mg/kg, IV). Heart rate was electronically derived from the electrocardiogram. Each bar represents the mean ± standard error of nine dogs.

dose in an anesthetized animal was a 9.6±2.2 mmHg increase in blood pressure. When the same dose of cocaine was administered after pretreatment with yohimbine, 0.25 mg/kg, the blood pressure response to cocaine was significantly increased to 20.6±2.8 mmHg. After pretreatment with atropine, 1 mg/kg, the blood pressure response to the 1 mg/kg dose of cocaine was increased to 17.6±3.5 mmHg. Pretreatment with both yohimbine and atropine resulted in a 44.2±7.2 mmHg elevation in blood pressure after administration of this same dose of cocaine.

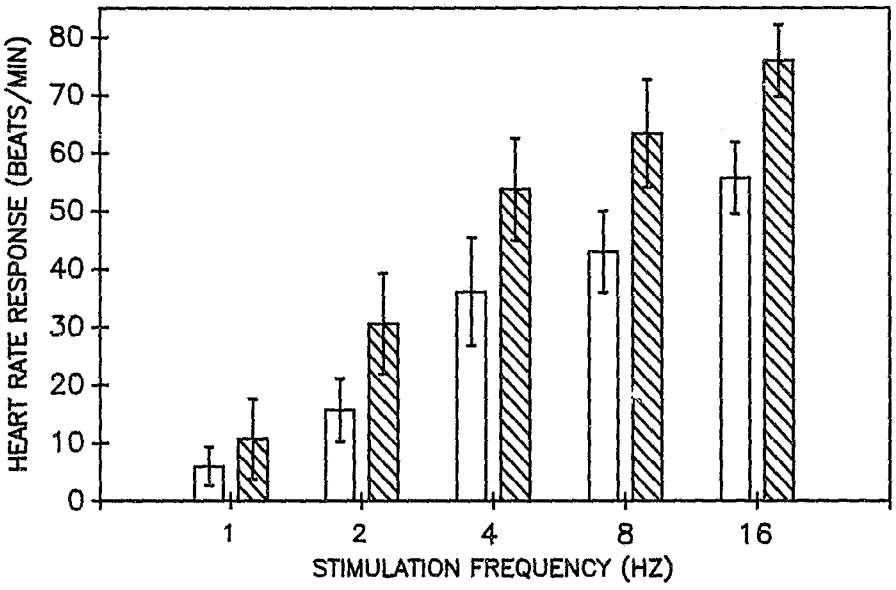

FIGURE 4. *Influence of pretreatment with cocaine (1 mg/kg, IV) on the heart rate response to cardiac sympathetic nerve stimulation in anesthetized dogs*

NOTE: The left ansa subclavia was electrically stimulated at the frequencies shown on the abcissa prior to (open bars) and after (hatched bars) cocaine administration. Heart rate was electronically derived from the electrocardiogram. Each bar represents the mean ± standard error of nine dogs.

In this same study, each animal was also instrumented for the measurement of myocardial contractile force using a Walton-Brodie strain gauge arch, which was sutured to the lateral wall of the left ventricle. The segment of myocardium subtended by the strain gauge arch was stretched incrementally to the peak of its length-tension curve by adjusting the distance between the feet of the arch. Thus, this segment of muscle contracted isometrically and, therefore, its force of contraction was not influenced by peripheral vascular alterations, which resulted in changes in ventricular preload or afterload. In addition, a pulsed doppler blood flow probe was placed around the left circumflex coronary artery. This probe allowed the measurement of the velocity of blood flow through its lumen utilizing the doppler shift principle. Each blood flow probe was precalibrated

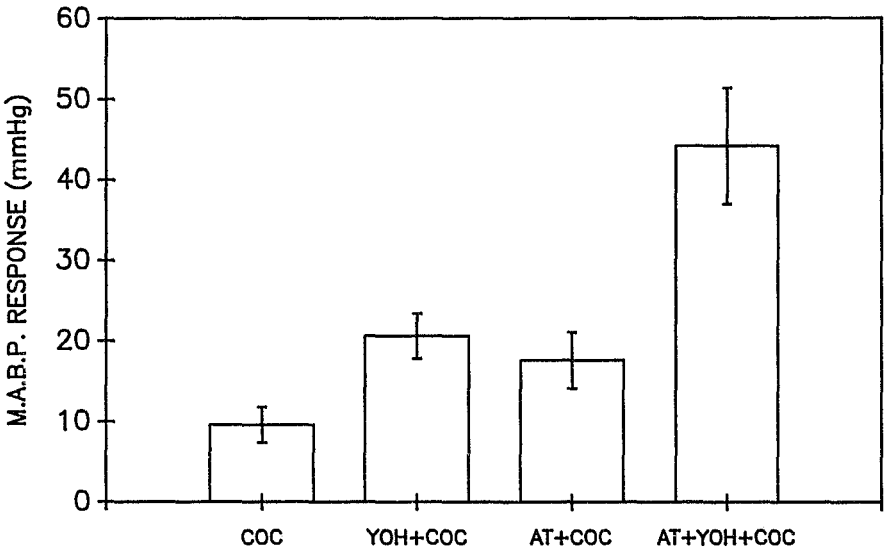

FIGURE 5. *Modification of the blood pressure response elicited by cocaine (1 mg/kg, IV) in anesthetized dogs by pretreatment with yohimbine (0.25 mg), atropine (1 mg/kg), or yohimbine plus atropine in these same doses*

NOTE: The left bar depicts the blood pressure response to 1 mg/kg of cocaine when given without any pretreatment, the left-center bar depicts the blood pressure response to the same dose of cocaine when administered after yohimbine pretreatment, the right-center bar depicts the response to the same dose of cocaine when administered after atropine pretreatment, and the right bar depicts the blood pressure response to cocaine when administered after pretreatment with both yohimbine and cocaine. Each bar represents the mean ± standard error of seven dogs.

to allow conversion of the measured doppler frequency shift to blood flow units (i.e., ml/min).

Coronary blood flow is typically regulated very effectively to match the heart's demand for oxygen and/or nutrients. This regulation of blood flow is the result of adjustments in the caliber of coronary arterioles (i.e., changes in resistance). Typically, in response to increased demand for oxygen and/or nutrients, coronary arteries dilate to increase blood flow to meet that demand. An example of

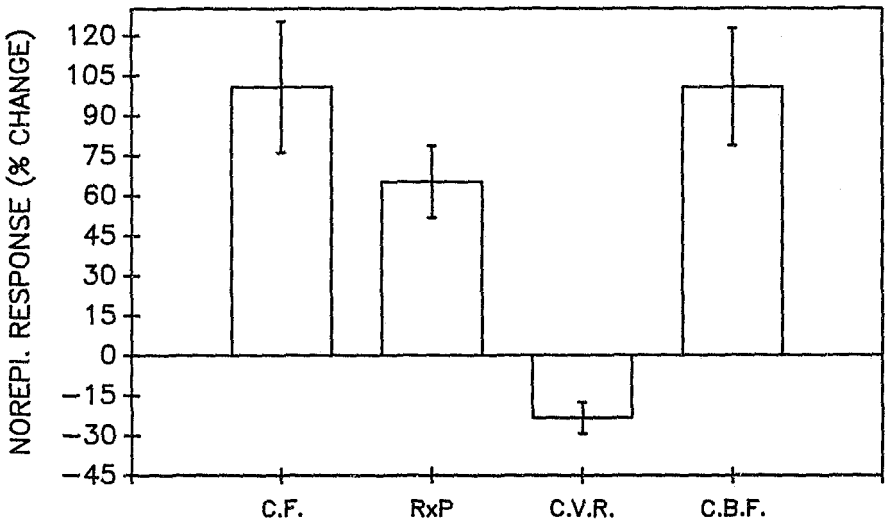

FIGURE 6. *Coronary vascular response to increased myocardial oxygen consumption elicited by administration of 0.25μg/kg of norepinephrine to anesthetized dogs*

NOTE: The left bar depicts the effects of norepinephrine (norepi.) on left ventricular contractile force (C.F.), the left-center bar depicts the effects of norepinephrine on the product of heart rate and blood pressure (RxP). The right-center and right bars illustrate the coronary vascular response to this change in myocardial oxygen demand. The right-center bar illustrates the decrease in coronary vascular resistance (C.V.R.), which resulted in the increase in coronary blood flow (C.B.F.) shown in the right bar. Each bar represents the mean ± standard error of six dogs.

this phenomenon in the preparation described above is illustrated in figure 6. When norepinephrine is administered at a dose of 0.25μg/kg, myocardial contractile force and the product of heart rate and blood pressure are increased, thus increasing myocardial oxygen consumption. This increase in the demand for oxygen is met by an increase in coronary blood flow, which is the result of a reduction in coronary vascular resistance (i.e., coronary vasodilation).

Figure 7 illustrates the effects intravenous cocaine, 1 mg/kg, on myocardial contractile force, the product of heart rate and blood

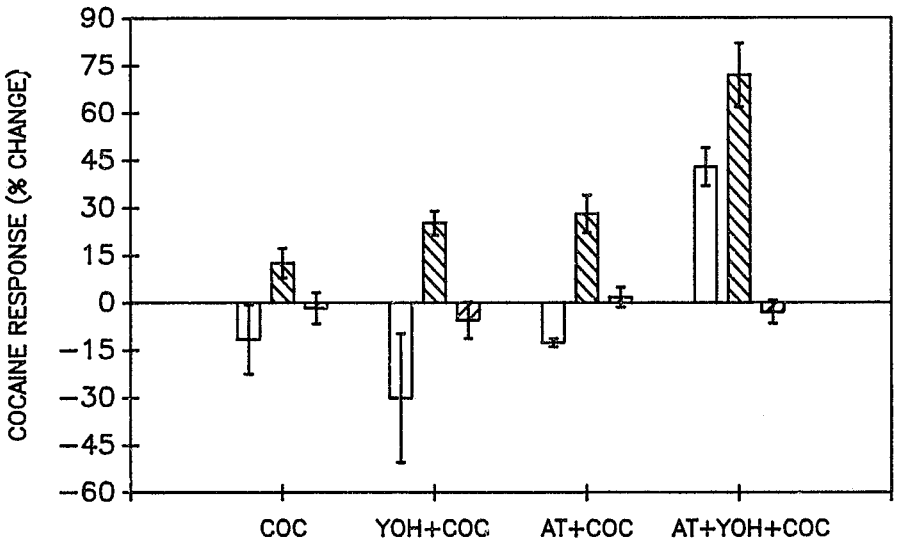

FIGURE 7. *Effects of 1 mg/kg of cocaine on left ventricular contractile force (open bars), the product of heart rate and blood pressure (right-slant hashed bars), and the coronary vascular response (left-slant hashed bars) to the change in myocardial oxygen consumption elicited by these effects of cocaine in anesthetized dogs*

NOTE: Cocaine was administered alone and after pretreatment with yohimbine (0.25 mg/kg), atropine (1 mg/kg), or a combination of yohimbine and atropine in these same doses. Each bar represents the mean ± standard error of seven dogs.

pressure, and coronary vascular resistance in anesthetized dogs instrumented as described above. When cocaine was administered alone and after pretreatment with yohimbine or atropine, it decreased myocardial contractile force but increased the rate-pressure product. Thus, in each case, it elicited effects that increased myocardial oxygen consumption (i.e., an elevation in the rate-pressure product) and other effects that decreased myocardial oxygen consumption (i.e., a reduction in contractile force). The reduction in myocardial contractile force was presumably the result of the local anesthetic actions of cocaine. However, when cocaine was administered after pretreatment with a combination of yohimbine and atropine, dramatic

increases in both myocardial contractile force and the rate-pressure product were observed. Clearly, in this latter instance, myocardial oxygen consumption was greatly increased, but this did not result in the expected decrease in coronary vascular resistance, which would be required to support an increase in coronary blood flow sufficient to meet the requirements of the heart during this response to cocaine. This apparently inappropriate response of the coronary vasculature to elevated myocardial oxygen demand elicited by cocaine administration suggests that cocaine may exert an action on the coronary circulation to prevent its normal dilation in response to increased oxygen demand. This finding, although not evidence of coronary vasospasm, clearly suggests that cocaine modifies the ability of the coronary circulation to dilate appropriately in response to normal physiologic stimuli (i.e., increased myocardial oxygen demand). These data further suggest that synaptic mechanisms that control the rate of norepinephrine release modulate the cardiovascular effects of cocaine and that any pharmacologic influence that interferes with these control mechanisms might be expected to significantly enhance its cardiovascular actions.

More comprehensive studies of the effects of cocaine on cardiovascular function in animals have also been performed, but these studies are few in number and all were performed in anesthetized animals or isolated hearts where the component of cocaine's cardiovascular action that is dependent upon central nervous system stimulation is absent or diminished. Pierre et al. (1985) have shown that a single large intravenous dose (200 mg) of cocaine in dogs results in a reduction in arterial blood pressure, cardiac output, and coronary blood flow.

Catravas and coworkers have reported two studies of cocaine toxicity in conscious dogs (Catravas et al. 1978; Catravas and Waters 1981). All animals in both studies exhibited cardiovascular effects of cocaine (e.g., increased blood pressure and heart rate). Although the precise role of the cardiovascular effects of cocaine in the lethality of this drug when infused at a rate of 0.5 mg/kg/min until death in dogs was unclear, these workers observed that the only interventions capable of protecting all animals from a 39.5 mg/kg dose of cocaine (three standard deviations above their previously determined mean lethal dose) were chlorpromazine pretreatment and reduction of ambient temperature to -5 °C.

The conscious dog is quite responsive to the cardiovascular effects of cocaine. In this model, mean blood pressure was elevated 95.8±11.0

mmHg after a single 8 mg/kg dose, and smaller cocaine doses also elicited dose-dependent increases in blood pressure (figure 8). Dogs with chronically implanted arterial and venous catheters were given cocaine intravenously in a range of doses (0.063, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg) to establish the dose-response relationship for this drug on blood pressure in this model. The data for this dose-response curve were obtained over a period of 8 days with only one cocaine dose being administered each day. Convulsions were observed in all animals after administration of the 8 mg/kg dose of cocaine, but no convulsions were noted at doses of 4.0 mg/kg or less.

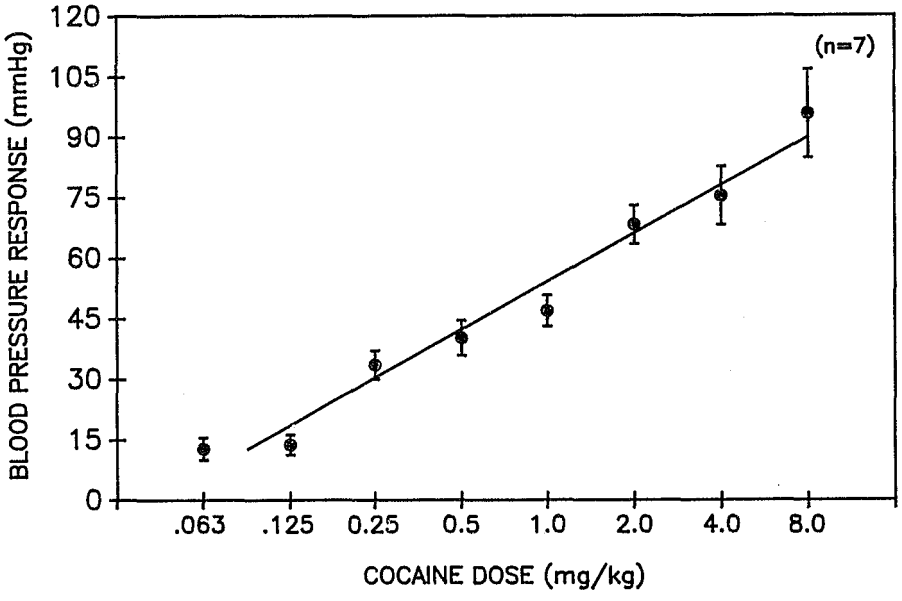

FIGURE 8. *Dose-response relationship for the effects of cocaine on blood pressure in trained, conscious dogs*

NOTE: The responses depicted were obtained when each dose of cocaine was administered as a single intravenous bolus with a minimum interval of 24 hours between doses. Each point represents the mean ± standard error of seven dogs.

Figure 9 illustrates the dose-dependent increase in the rate-pressure product observed after administration of increasing doses of cocaine on different days in conscious dogs instrumented for the measurement

of blood pressure, heart rate, and coronary blood flow as described above. At all doses studied, cocaine administration resulted in a significant increase in the rate-pressure product, and, at doses above 2 mg/kg, this index of myocardial oxygen consumption was more than doubled. As described above, typically, increases in myocardial oxygen demand are appropriately matched by coronary vascular dilation in order to meet this increased need for oxygen and nutrients. At all doses of cocaine studied, coronary vascular resistance increased despite apparent increases in myocardial oxygen demand. Thus, in the conscious dog model, there appears to be evidence

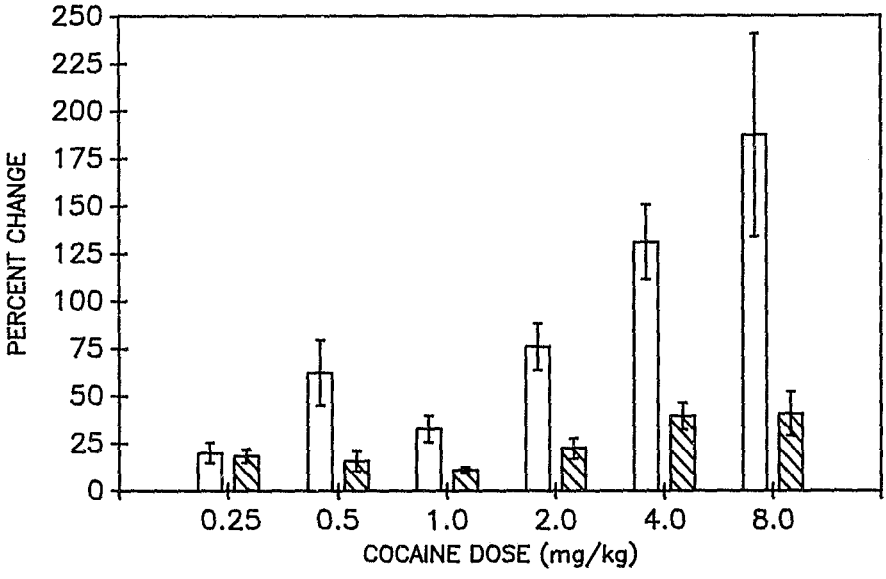

FIGURE 9. *The effects of cocaine on myocardial oxygen consumption and coronary artery function in trained, conscious dogs*

NOTE: Cocaine was administered in the doses shown with a minimum of 24 hours between doses. The product of heart rate and blood pressure (open bars) was utilized as an index of myocardial oxygen consumption, and coronary vascular resistance (hatched bars) was calculated from arterial blood pressure and left circumflex coronary artery blood flow. Each bar represents the mean ± standard error of seven dogs.

for an action of cocaine to modify normal coronary function. Whether or not the observed inappropriate increase in coronary vascular resistance, in the face of increased myocardial oxygen

demand, is sufficient to produce myocardial infarction is an open question. It does seem quite clear, however, that cocaine exerts an action to interfere with the normal control of coronary blood flow. Such an action could have disastrous consequences in individuals whose ability to increase myocardial blood flow in response to an increase in myocardial oxygen demand is already compromised by atherosclerotic changes in their coronary vessels. Even in individuals with normally functioning coronary arteries, the actions of cocaine to increase myocardial oxygen demand, while interfering with the normal blood flow regulation in the heart, could conceivably result in myocardial ischemia.

There have been several studies of the cardiovascular effects of cocaine in human subjects who were cocaine users. These subjects received intravenous doses of cocaine ranging from 4 to 48 mg and typically exhibited dose-related increases in heart rate and blood pressure (Fischman et al. 1976; Resnick et al. 1977; Javaid et al. 1978; Fischman et al. 1983). To put these doses in perspective, Fischman and Schuster (1982) asked their subjects to compare the cocaine doses received to their typical "street" dose. A dose of 16 mg was generally rated as similar to their average "street" dose, while doses of 24 and 32 mg were rated as among the highest those subjects had ever experienced.

Recently, Fischman et al. (1985) reported that acute tolerance develops to the tachycardia associated with cocaine administration in humans. When subjects were allowed to self-administer 96 mg of cocaine intranasally 1 hour prior to an intravenous dose of cocaine, the heart rate response to the intravenous dose was significantly reduced in comparison to the response to the same intravenous cocaine dose in the same subjects 1 hour after intranasal administration of a placebo. Although the increments in plasma cocaine concentration produced by similar intravenous doses were not significantly different in the two groups, the residual plasma cocaine concentration remaining from the intranasal cocaine dose was approximately 160 ng/ml at the time of intravenous cocaine administration in the one patient whose plasma cocaine concentrations were presented. The authors interpreted these findings to be indicative of acute tolerance to the actions of cocaine. Clearly, these results demonstrate that, when cocaine is present in the body at the time of administration of an acute intravenous cocaine dose, the heart rate response to that dose is less than when the same intravenous dose is administered in the absence of residual plasma cocaine. Whether this is the result of functional tolerance, or merely

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due to the fact that the dose (concentration)-response relationship for cocaine, like most drugs, is not linear but rather forms a rectangular hyperbola is unclear. That is, the "dose-response" curve is linear only when response is plotted as a function of log dose. Thus, a 450 ng/ml increment in plasma cocaine concentration would be expected to produce a greater response, for example, when it occurs from 0 to 450 ng/ml than when it occurs from 160 to 610 ng/ml. In this regard, it would be interesting to know whether or not the reduced responsiveness to the intravenous cocaine dose would occur if it were administered a sufficient time after the intranasal cocaine dose for the residual plasma cocaine concentration to fall to a negligible level. Similarly, it is important to determine whether this reduced responsiveness is peculiar to heart rate or whether it also occurs with other cardiovascular parameters. The very efficient control of heart rate by baroreceptor reflexes makes heart rate a particularly complex parameter to use to evaluate tolerance, since a major component of the cardiovascular response to cocaine is an increase in arterial blood pressure. Finally, as pointed out recently by Porcet and his colleagues (1987), when the concentration of a drug that distributes rapidly to its site of action cannot be measured at that active site, it may not be possible to assess acute tolerance by bolus-dose experiments alone. Steady-state experiments may also be required to distinguish acute functional tolerance from apparent tolerance resulting from distributional differences with drugs such as cocaine or nicotine, which distribute very rapidly into the brain. Matsuzaki and coworkers (1978) also reported the development of tolerance to the cardiovascular actions of cocaine after chronic administration, but they also reported that chronic cocaine treatment decreased the elimination half-life of cocaine by almost 50 percent. This suggests that the reduced response to cocaine that they observed may have been the result of lower cocaine plasma concentrations resulting from its faster metabolism rather than from a true functional tolerance. Unfortunately, they did not measure cocaine plasma concentrations in conjunction with their physiological measurements. Whether or not acute functional tolerance actually occurs to the cardiovascular effects of cocaine is uncertain at this time, but because of this uncertainty we have modified our acute study protocols so that no more than one cocaine dose is administered during any 24-hour period.

In conclusion, despite a lack of concrete evidence demonstrating cardiovascular toxicity of cocaine in man, there is a growing volume of circumstantial evidence that suggests that cardiac and vascular toxicity associated with cocaine use may lead to myocardial infarction

and stroke. These data are complemented by observations in conscious animal preparations that cocaine increases myocardial oxygen consumption, as measured by the product of heart rate and blood pressure, while simultaneously interfering with metabolic vasodilation in the coronary circulation. Indeed, there is an inappropriate increase in coronary vascular resistance as a result of cocaine treatment. Clearly, more studies are needed to better define the effects of cocaine on the cardiovascular system, but because of the complex effects of cocaine on the central nervous system and on the heart and blood vessels, the integrated effects of cocaine on the cardiovascular system can only be studied appropriately in the intact, fully conscious animal.

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Arteriosclerotic Toxicity of Cocaine

Ronald O. Langner, Collette L. Bement, and Lynda E. Perry

INTRODUCTION

The purpose of our research is to test the hypothesis that cocaine is a potential risk factor for the premature development of arteriosclerosis. The abuse of cocaine has been associated with a number of cardiovascular toxicities including tachycardia, hypertension, ventricular arrhythmias, and death. In small doses, cocaine can depress the heart rate as a result of central vagal stimulation. When cocaine is used intravenously in high concentrations, it produces a dose-related increase in heart rate and blood pressure (Fischman et al. 1976). These effects are due both to central sympathetic stimulation and the peripheral effects of cocaine in inhibiting the reuptake of catecholamines at adrenergic nerve endings. The resulting increased sympathetic tone leads to tachycardia and raises the blood pressure, which in turn increases the oxygen demand of the heart. This sequence of events has been suggested as a possible cause of myocardial infarctions observed in some individuals following the abuse of cocaine (Ring and Butman 1986).

Cardiovascular disease remains the leading cause of death in the United States and Western Europe. Arteriosclerosis, which accounts for the majority of these deaths, is a group of diseases characterized by increased collagen deposition and loss of elasticity in the arterial wall. Atherosclerosis, which is a form of arteriosclerosis, is characterized by the presence of yellowish plaques (atheromas) containing cholesterol, as well as by the loss of elastin and deposition of collagen. These lesions are normally found in the intima and media of large- and medium-sized arteries. The earliest arteriosclerotic lesions are thought to begin during the first decade of life (McGill 1984) and may or may not contain increased amounts of cholesterol. During the

third and fourth decades of life, fibrous plaques with necrotic, lipid-rich cores surrounded by smooth muscle and connective tissue appear. These lesions undergo vascularization, hemorrhage, and ulceration, and some eventually are covered by thrombi, which occlude the artery and cause ischemic necrosis in the affected tissue. Even though the clinical manifestations of arteriosclerosis vary with the location of the lesions, all the underlying arterial lesions are essentially the same.

Many risk factors have been associated with the development of arteriosclerosis. Hopkins and Williams (1981) listed 246 different factors that have been implicated in the development of the disease. These were divided into primary and secondary risk factors with elevated serum cholesterol, hypertension, cigarette smoking, and obesity being considered as primary risk factors. In a recent review of the mechanism of action in atherogenesis, Ross (1986) suggests that all atherosclerotic risk factors cause an injury to the blood vessel wall and represent some of the earliest events in lesion formation. When the injury is recurrent, the resulting damage to the arterial lining stimulates migration and proliferation of smooth muscle cells to the damage site. In the presence of moderate to elevated serum cholesterol, smooth muscle cells are thought to accumulate large amounts of cholesterol, resulting in cellular necrosis. This is further complicated by a buildup of fibrous tissue (mostly collagen) and calcium leading to a clinically significant narrowing of the arteries.

Several investigators have demonstrated that intravenous injection of epinephrine results in the development of arteriosclerotic lesions, which are similar to those found in the early stages of human arteriosclerosis (Oester 1959; Lorenzen 1961). The lesions are primarily localized in the thoracic aorta and the ascending and descending portions of the aortic arch. The histological changes induced in the aorta were primarily in the medial layers, with some changes in the intima, and were characterized by damage or necrosis of the inner arterial walls, breakdown, splitting, and eventual disappearance of the elastic lamella and an infiltration of the injured area with water, proteins, and glycosaminoglycans. Surrounding this necrotic area are layers of smooth muscle cells, which appear to be active both as fibroblasts and phagocytes. Calcification of the injured area seems to be a secondary event, which follows the deposition of glycosaminoglycans. These epinephrine-induced lesions do not exhibit increased lipid accumulation. However, experimental studies by Constantinides et al. (1958) have shown that arteries injured by epinephrine will

incorporate lipids when rabbits are fed a high cholesterol diet either during or after epinephrine treatment.

Fuller and Langner (1970), studying the biochemical changes induced by epinephrine treatment, found that one of the earliest changes was an increase in the rate of aortic collagen synthesis. This change was observed after only 4 days of treatment and before any gross alterations in aortic morphology were noted. The authors suggested that changes in aortic collagen synthetic rates represented a primary biochemical defect. The mechanism whereby epinephrine causes these changes is unknown. Waters and de Suto-Nagy (1950) suggested that the hypertensive effect of epinephrine caused an injury to the endothelial lining, which was responsible for the observed changes. Since cocaine exerts its cardiovascular effects by potentiating the effects of epinephrine and norepinephrine, these studies were initiated to establish the potential of intravenous cocaine to cause an arteriosclerotic injury to the vessel wall.

METHODS

Fifteen male New Zealand rabbits weighing 2.8 to 3.2 kg were used in these studies. Five rabbits were placed in a saline control group and the remaining 10 rabbits were placed in a drug treatment group. All animals were given standard rabbit chow and water *ad libitum*. Each rabbit was weighed daily and injected via the marginal ear vein for 14 days using an infusion pump set to deliver fluid at a rate of 1 ml per minute. The saline control group received injections of sterile saline for 1 minute. The cocaine-treated rabbits were injected with the following doses of drug: days 1 and 2, 5.5 mg/kg; days 3 through 10, 4.5 mg/kg; and days 11 through 14, 5.0 mg/kg.

The initial dose of 5.5 mg/kg was selected following preliminary studies that indicated that this dose of cocaine would result in an elevation in the heart rate. After 2 days, the dose of cocaine was decreased to 4.5 mg/kg, because the initial dose of cocaine resulted in the death of two rabbits and visibly stressed the remaining animals. After 10 days, the dose was increased to 5.0 mg/kg, because the rabbits were exhibiting little visible response to the cocaine, suggesting that some degree of tolerance was developing.

Twenty-four hours after their last injection, the rabbits were lightly anesthetized with ether and killed by cervical dislocation. The thoracic aorta and the liver were quickly removed and placed in cold Kreb's bicarbonate buffer (pH 7.4). The thoracic aorta was cleaned

of loosely adhering fat and tissue, opened longitudinally, and inspected for gross aortic lesions. Aortic samples from the descending aortic arch were removed for histological evaluation. The liver was handled in a similar manner. The aorta and liver were then placed in fresh buffer containing 3 uCi of ^{14}C-proline and 0.2 mM L-proline for either 90 minutes (aorta) or 60 minutes (liver), following procedures previously reported (Langner and Bement 1985).

Following the incubation period, the tissues were rinsed in 5 percent ice-cold trichloroacetic acid (TCA) to prevent further incorporation of the labeled proline. The tissues were then homogenized in 9 volumes of normal saline using a glass coaxial homogenizer. Appropriate aliquots were taken for analysis of collagen hydroxyproline content (Kivirrikko et al. 1967), protein content (Lowry et al. 1951), cholesterol content (Franey and Amador 1968), and protein synthetic rate (Langner and Bement 1985). For histological evaluation, all samples were fixed in 10 percent formalin and were stained with hematoxyline and eosin, and Verhoeff's. The data were evaluated for statistically significant differences using the Student's t-test (Snedecor and Cochran 1976).

RESULTS

As shown in table 1, the cocaine treatment did not cause any alteration in serum cholesterol levels. The final body weights of the

TABLE 1. *Effect of cocaine on final body weight and serum cholesterol levels*

Group	N	Final Serum Cholesterol (mg/100 ml)	Final Body Weight (kg)
Saline	5	44.12±7.74	3.46±0.2
Cocaine	8	49.14±5.89	2.90±0.1*

*p<.05 compared to saline-treated controls.

NOTE: Data expressed as mean ± SE.

FIGURE 1. *Aorta from a rabbit treated for 14 days with cocaine, showing general disruption of elastic fibers*

NOTE: The spaces between the elastic fibers appear widened with increased deposition of granular material. (Verhoeff's, 250X.)

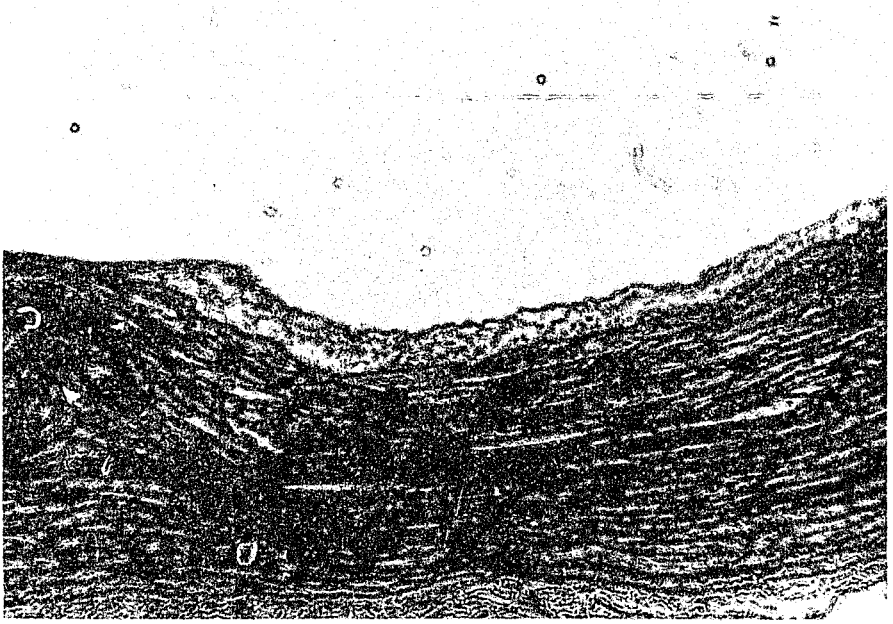

FIGURE 2. *Aorta from a rabbit treated for 14 days with cocaine, showing a compression of the medial layer*

NOTE: The intimal layer is thickened and appears to contain increased numbers of foam cells. (Verhoeff's, 250X.)

cocaine-treated rabbits were significantly lower than the saline-treated controls. Visual inspection of the aorta revealed that five of the eight cocaine-treated animals had some focal lesions, primarily in the area of the upper thoracic and aortic arch. These lesions were small and raised and had a pearly white appearance. There were no observable lesions in the saline-injected controls.

Histological evaluation of the aortic lesions (figures 1 and 2) demonstrated that the cocaine-induced lesions could be quite different. In figure 1, the lesion appears to be primarily located in the media with little evidence of intimal involvement. The lesioned area is characterized by edema and widening of the spaces between the elastic lamella. In addition, the elastic fibers show fragmentation and general disruption. Within the widened spaces there appears to be increased deposition of a granular exudate. In figure 2, the lesion is much different and shows a compression of the media with little

rearrangement or damage. In this lesion, however, there is intimal proliferation over the area of compressed media, which appears to have a foam-cell-like appearance. Histological evaluation of the livers from cocaine-treated rabbits did not exhibit any pathological alterations.

The data in table 2 show the tissue body weight ratios of both the liver and thoracic aorta. As can be seen, the administration of cocaine for 14 days did not cause any change from control values even though the total body weights of the cocaine-treated rabbits were less than the saline animals (table 1). There also was no change in tissue cholesterol content in either the aorta or the liver.

TABLE 2. *Tissue to body weight ratios and total cholesterol content of thoracic aortas and livers of rabbits treated with cocaine*

	Thoracic Aorta		Liver	
	Tissue/Body	Cholesterol	Tissue/Body	Cholesterol
Group	(g/kg)	(mg/g tissue)	(g/kg)	(mg/g tissue)
Saline	0.25±.02	3.94±.52	19.40±1.37	4.48±.16
Cocaine	0.28±.02	3.83±.39	18.37±0.59	4.38±.16

NOTE: Data expressed as mean ± SE.

The total collagen content in both the aorta and liver were estimated by measuring tissue hydroxyproline content. Hydroxyproline is primarily found in collagen. Smaller amounts of hydroxyproline are also found in elastin and the C1q component of complement. As seen in table 3, the administration of cocaine did not cause any significant alteration in either aortic or liver hydroxyproline content. The protein content of both the aorta and liver also were not altered by the administration of cocaine for 14 days.

The data in table 4 show that there was a significant increase in the rate of protein synthesis in the thoracic aortas of rabbits treated with cocaine, as indicated by a threefold increase in the rate of ^{14}C-proline incorporation into aortic proteins. The livers of the cocaine-treated rabbits did not demonstrate any change in protein synthetic

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activity in response to the cocaine administration. These data, therefore, suggest a relatively specific response of the blood vessel wall to the administration of cocaine.

TABLE 3. *Hydroxyproline and protein content of thoracic aortas and livers of rabbits treated with cocaine*

	Thoracic Aorta		Liver	
Group	Hydroxyproline (μg/g)	Protein (mg/g)	Hydroxyproline (μg/g)	Protein (mg/g)
Saline	7696±394	104±11	737±81	169±4
Cocaine	7520±460	98±9	810±89	174±2

NOTE: Data expressed as mean ± SE.

TABLE 4. *Protein synthetic activity of thoracic aortas and livers of rabbits treated with cocaine*

Group	Thoracic Aorta dpm/mg Protein 90 min	Liver dpm/mg Protein 60 min
Saline	226.6±60	199.5±35
Cocaine	688.7±165 *	282.7±29

*p<.05 compared to saline controls.

NOTE: dpm of ^{14}C-proline incorporated into either aortic or liver proteins per milligram of tissue protein. All data expressed as mean ± SE.

DISCUSSION

The purpose of our studies was to determine the potential of chronic cocaine administration to induce early arteriosclerotic changes in the blood vessels of rabbits. In humans, arteriosclerosis frequently begins in the form of scattered foci in which the innermost layers of the vascular wall show signs of damage, accompanied by the growth of

repair tissue (Constantinides 1965; Daoud et al. 1964). Injury is characterized by the disruption and disintegration of the innermost elastic lamella and by an increase in amorphous materials, particularly proteins and glycosaminoglycans. At this early lesion stage there is not an abnormal amount of cholesterol. Lipids appear to accumulate slowly until the lesions become grossly visible and are observed as fatty streaks (Constantinides 1965). The histological changes we observed in the cocaine-treated rabbits appear to be similar to early human lesions described by Constantinides (1965). The lesions are primarily located in the medial layers of the aortic wall and may or may not be grossly visible. These early cocaine-induced lesions do not contain elevated levels of cholesterol (table 2) and do not appear to be the result of any alteration in serum cholesterol levels (table 1). The observed increase in aortic protein synthetic rates (table 4) suggests that the tissues are in a proliferative stage of development. The absence of any change in total collagen or total proteins may be a result of the lesions being in an early stage of development. At this stage of development, sufficient time may not have elapsed to allow for a detectable accumulation of these proteins. Another possibility is that cocaine treatment has induced both synthesis and degradation of proteins, resulting in no net accumulation of either collagen or total proteins.

The changes induced by cocaine administration were found only in the aorta and not in the liver. Histological and biochemical examination of liver sections revealed no significant alterations. These data would suggest that the induction of aortic protein synthetic rates is the result of a direct change in the aortic wall and not the result of some generalized increase in body protein synthetic activity. When cholesterol is used to induce atherosclerotic lesions in rabbits, both the aorta and the liver have markedly elevated tissue cholesterol levels and increased rates of protein synthetic activity (Langner and Modrak 1981). The ability of cocaine to induce arteriosclerotic changes appears to be more specific in that only the aortic wall is affected. The mechanism by which cocaine produces its effect is not known. The biochemical and histological changes produced by cocaine administration, however, are very similar to the changes observed following epinephrine administration (Langner and Fuller 1973). It has been suggested that epinephrine administration causes an increase in blood pressure, which in some manner may cause an injury to the intimal lining of the blood vessel (Waters and de Suto-Nagy 1950). Since cocaine has the ability to potentiate endogenous catecholamine activity, the arteriogenic effect of cocaine may be the result of increased catecholamine activity resulting in an increase in heart rate

and blood pressure. The exact mechanism by which increases in blood pressure and heart rate damage the arterial wall is unknown and needs to be investigated.

The significance of our observations in rabbits to the development of arteriosclerotic disease in man remains to be elucidated. Further experiments using different doses of cocaine for varying times must be investigated. In addition, the potential for cocaine to act synergistically with other known cardiovascular risk factors to cause premature atherosclerosis should also be studied. Our data do, however, clearly demonstrate that cocaine has the potential to cause an injury to the blood vessel wall that may result in arteriosclerotic-like changes.

SUMMARY

The repeated injection of cocaine results in the formation of arteriosclerotic lesions in rabbits, which appear to be similar to early arteriosclerotic lesions seen in man. Biochemically, the aortas exhibited increased rates of protein synthesis, without any change in tissue cholesterol content. The cocaine-treated rabbits exhibited no change in serum cholesterol levels. These data suggest that the abuse of cocaine may result in damage to the aorta, which could result in the premature onset of cardiovascular disease and its complications.

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Mechanisms of Cocaine Abuse and Toxicity: An Overview

Thomas V. Dunwiddie

INTRODUCTION

It is characteristic of pharmacology that drugs are often initially perceived in terms of their beneficial or therapeutic uses, and only after they have come into common use by a large number of individuals do the drawbacks and difficulties attendant upon their use become known. Certainly this has been the case with cocaine. Initially introduced and popularized for various purposes such as increasing alertness, elevation of mood, overcoming opiate addiction, etc., it became clear even during the last century that cocaine overuse had the potential for severely disrupting both physical and psychological well-being. The neurobiological basis for the abuse potential of cocaine was identified in a general sense by Sigmund Freud, who did much to initially popularize the drug. Freud suggested that the feelings of pleasure and well-being that accompanied the use of cocaine were quite similar to those engendered by many other types of stimuli in daily life that give pleasure. Now, with over a century of neurobiological research to lend further insight, it is apparent that the ability of cocaine to so potently disrupt normal activities stems from the fact that it can interact directly with the same brain systems (sometimes called "reward" pathways) that are normally activated indirectly by pleasurable stimuli. In a sense, cocaine can "short-circuit" the means by which gratification is usually achieved, and the typical pleasurable activities (e.g., eating, drinking, sexual behaviors, etc.) become less satisfying. For both the drug abuser of the 1980s and for laboratory animals in drug self-administration protocols, cocaine acts as a potent reinforcer that supersedes nearly all other kinds of reinforcement as a determinant of behavior.

Unfortunately, it is clear that simply understanding the biological bases for specific maladaptive behaviors does not immediately dictate their solution. In fact, given our current understanding of the biological underpinnings of drug self-administration behavior, one might well adopt a more pessimistic view of the likelihood of developing pharmacological treatments for substance abuse. If drugs such as cocaine directly activate neural substrates of reward, is it even possible to develop pharmacological treatments that could block pharmacological reward without severely disrupting normal appetitive behaviors? At this point, it is perhaps premature to try to answer this question. Nevertheless, as we begin to understand more about the basic biology of drug abuse, it is clear that our attempts to control such behaviors can shift from the purely empirical, to strategies that are directed by an understanding of the neural systems involved. Many of the papers in this monograph contribute substantially to this understanding, and it seems probable that the development of more successful treatments for drug abuse may eventually develop out of the basic science presented at this meeting.

When considering the recent research advances in this field, it is difficult to point to any specific observations as being "key" advances in our understanding of drug abuse. What is apparent, however, is that, at many different levels of inquiry, a simplistic model concerning the actions of cocaine is being replaced by a more detailed and necessarily more complicated picture of action. The original pharmacological picture of cocaine was that of a local anesthetic, which upon further study was also found to be an inhibitor of the uptake of the neurotransmitter norepinephrine. Now, however, we know that cocaine inhibits not only the uptake of norepinephrine but also that of dopamine and serotonin. Furthermore, the work of several participants in this review (Reith, this volume; Hanbauer, this volume) suggests that high-affinity dopamine uptake, which until now has been regarded as a unitary process, may represent multiple processes with independent pharmacological properties.

In a similar vein, some of the earlier pharmacological studies of drug self-administration suggested that dopamine was centrally involved in such behaviors as "the" reward transmitter. However, it is difficult now to regard pharmacological reinforcement as a monolithic "system," in which the mere occupation of a dopamine receptor somehow can be equated with positive reinforcement. Instead, it is clear that a drug such as cocaine, which causes an inhibition of dopamine uptake, elicits a complex range of effects on dopaminergic systems. Moreover, it is likely that a variety of transmitter systems

(opioid, cholinergic, and most likely peptides such as neurotensin and cholecystokinin as well) may also be involved in ways that remain undetermined. As was suggested by several participants at this conference, reward systems may have distinct initiation, mediation, and presumably termination components, and pharmacological agents might interact with any one of these components to modify drug-seeking and self-administration behaviors. Similarly, when dealing with drugs that are self-administered, as well as with drugs that disrupt such behaviors, one cannot focus simply upon the rewarding aspects of these drugs, but must also take into account their effects upon motor systems and their abilities to act as discriminative cues. It seems likely that only by subdividing every aspect of cocaine's actions (drug self-administration into its component behavioral processes; the biochemical actions of cocaine into effects upon different transmitters, ion channels, receptors, and uptake sites) will we be able to fully understand the way in which substance abuse evolves.

RECENT PROGRESS IN UNDERSTANDING THE ACTIONS OF COCAINE

Biochemical Aspects of Cocaine Action

The last decade has seen a remarkable change in the level of sophistication at which we understand the interactions of transmitters with receptors and the secondary processes by which receptor activation by a transmitter is translated into the physiological responses of neurons to such transmitters. However, our understanding of drugs that interact with more peripheral aspects of neurotransmission, such as allosteric regulation of transmitter binding, neurotransmitter uptake, etc., has perhaps lagged. In part, this is due to the fact that it is more difficult to measure physiological responses to these types of drugs; if a drug such as cocaine inhibits uptake, this does not usually lead directly to any well-defined physiological response, but only to indirect effects that represent the interaction of increased extracellular concentrations of transmitters with their receptors. Correspondingly, studies of cocaine binding in the brain have been hampered somewhat by the difficulties in linking specific binding sites to functional effects; although binding can be observed, it is often complex, and the physiological consequences of binding to these sites are often subtle.

One approach to these difficulties has been to correlate the relative potencies of drugs in terms of well-defined biochemical properties with their ability to elicit relatively complex behaviors such as drug

self-administration, without attempting to define the intervening physiological events. Kuhar adopted the approach of labeling specific neurotransmitter uptake sites with selective ligands, and then characterizing their displacement by a variety of abused and nonabused drugs (Kuhar et al., this volume; Ritz et al. 1987). Binding to the dopamine uptake site generally correlated well with the extent to which these drugs are self-administered in animals, whereas their ability to inhibit the uptake of serotonin and norepinephrine did not display such a relationship. These data provide further support for the hypothesis that potentiation of the effects of dopamine is intimately involved in pharmacological reward. In addition, these studies help to resolve the continuing dilemma of how to reconcile the "dopamine hypothesis" with the experimental observation that a number of local anesthetics are self-administered. The present findings imply that the reinforcing actions of some local anesthetics are a function of their actions upon the dopamine uptake site and that local anesthetic actions may be only peripherally involved. The use of newer pharmacological agents, such as the more selective uptake inhibitors, possibly irreversible inhibitors of uptake, and local anesthetics that have little or no effect upon uptake processes, may lead to an even more finely drawn portrait of the mechanisms by which monoamine uptake occurs, the ways in which cocaine can interact with such processes, and how this can lead to pharmacological reinforcement.

The experimental results discussed by a number of the participants also suggest that our current models of high-affinity uptake processes for monoamine transmitters need revision. The observations of Reith (this volume) made it clear that, although cocaine affects the uptake of norepinephrine, dopamine, and serotonin, it is not a particularly suitable ligand for characterizing the corresponding uptake sites. In particular, the binding of labeled cocaine appears to correspond in many regions with high-affinity uptake of dopamine and serotonin, but not norepinephrine, even though cocaine is able to inhibit uptake of norepinephrine quite well. Disparities also arose between some of the effects of metaphit (an irreversible acylator of PCP-binding sites) upon binding and behavior; for example, metaphit treatment *in vivo* blocked the locomotor stimulant effects of cocaine (but not indirect dopamine-releasing agents such as amphetamine), but did not affect either dopamine uptake or cocaine binding. The work of Hanbauer (this volume), who demonstrated that an unidentified endogenous peptide as well as GABA modulin are allosteric regulators of ^{3}H-cocaine binding, also suggests that the uptake processes may not be simple transporters, but may have a complex regulation by endogenous

agents that might be perturbed by cocaine. Clearly, a better understanding of the transport process per se will lead to a more detailed picture as to how cocaine acts as well.

Physiological Aspects of Cocaine Action

In terms of the electrophysiological actions of cocaine, there has been relatively little work done in comparison with other drugs of abuse such as amphetamine. Relatively recent reports have demonstrated that cocaine can inhibit the spontaneous firing of neurons in the locus coeruleus, the ventral tegmental area, and the dorsal raphe (White 1985; Pitts and Marwah 1986; Brodie and Dunwiddie 1986; Lakoski and Cunningham, this volume). In each case where this has been examined, the mechanism underlying this inhibition appears to be a facilitation of the actions of the endogenous monoamine transmitter (norepinephrine, dopamine, and serotonin, respectively) upon autoinhibitory receptors.

Two aspects of these responses will require further study. First, the general hypothesis of cocaine action postulates a facilitatory action of cocaine at monoaminergic synapses; however, if the neurons containing these transmitters are themselves inhibited by cocaine, then there is no release of transmitter to provide a substrate upon which cocaine can act. It is possible that cocaine in behaviorally active doses does not totally suppress presynaptic activity, but merely reduces it, and in this situation the net effect of cocaine administration could still be an enhanced postsynaptic effect. Alternatively, as White (1985) has demonstrated, the mesocortical dopamine neurons of the ventral tegmental area may be relatively insensitive to the impulse-inhibiting effects of cocaine, since they appear to lack the autoinhibitory receptors found on other ventral tegmental area neurons. Thus, cocaine might selectively enhance activity in mesocortical pathways while having a less pronounced effect upon the mesoaccumbens and other projections that have autoreceptors. The other aspect of the physiological effects of cocaine that needs further investigation concerns the relative sensitivity of different monoamine systems to cocaine and the ways in which these systems interact. Lakoski and Cunningham (this volume) presented evidence that the raphe nucleus appears to be the most sensitive of the monoamine-containing nuclei to the inhibitory effects of cocaine. This effect was shared by other serotonin uptake inhibitors such as fluoxetine, but not by selective dopamine and norepinephrine uptake inhibitors. Since there is behavioral evidence to suggest that serotonergic systems are not involved directly in the rewarding

effects of drugs such as cocaine (Koob and Hubner, this volume), it is possible that inhibition of serotonergic systems might well relate to some of the other, nonreinforcement properties of cocaine. Behavioral and electrophysiological studies involving selective serotonin uptake inhibitors such as fluoxetine and paroxetine may help to clarify these issues.

RELATING BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF COCAINE TO "REWARD"

Although the effects of cocaine can be studied independently from a consideration of pharmacological reward, at some point it becomes useful to try to relate specific changes in neuronal function to the psychological concept of reinforcement. A problem that arose repeatedly during this conference was the difficulty in quantifying "reward" per se. As was pointed out by several speakers (Balster, Winger, Dworkin, and Johanson), the rate of responding on a lever for drug administration is a clearly inadequate measure of the "potency" of a rewarding stimulus. Typically, animals will respond to increasing doses of drug with increasing response rates up to a point, but beyond that point responding drops off, so that at the highest doses response rates may be quite low. Nevertheless, when allowed to choose between a lower or higher dose of drug, animals typically select the higher dose, even though it is associated with lower rates of administration (Johanson, this volume). Several types of protocols have potential for determining "reward potency," such as choice studies, and so-called break-point studies, in which the measure of reward is determined by increasing the number of responses required of the animal for each successive injection. The point at which the animal stops working for a given dose of drug thus becomes the "break-point" for that dose.

Presumably this technique will lead to the characterization of drugs not only in terms of relative potency (i.e., the amount of drug required to maintain equivalent break-points) but also in terms of efficacy (i.e., the maximum break-point that can be established by any dose of the drug). The development of more unequivocal techniques such as these for characterizing the rewarding properties of drugs may lead to a better understanding of the neurobiological basis for reward as well. Dworkin and Smith (this volume) also discussed ways in which to dissociate the various pharmacological properties of cocaine, viz, the reinforcing effects, the effects upon response rate, and the stimulus properties. Different types of behavioral protocols are sensitive to each of these variables, and by suitable experimental

design, it is possible to fractionate the changes in behavior attributable to each of these actions of cocaine.

The approach used by Kuhar et al. (this volume) to identify binding sites in the brain that are related to the reinforcing properties of drugs is an important first step in determining how drugs such as cocaine act. With improving behavioral techniques for quantifying reinforcement, these correlations between biochemistry and behavior may become even tighter. However, it is also clear that defining reinforcement by means of a single biochemical parameter may be too simplistic. As was pointed out by Balster (this volume), in terms of reward, cocaine and amphetamine appear quite similar, although biochemically they probably act through quite different mechanisms. On the other hand, other drugs that share some of cocaine's actions appear quite different in behavioral studies. There are a number of possible explanations for this anomaly. First, it is clear that the pharmacokinetics of the drug response is an important variable; Johanson (this volume) demonstrated that the rate of drug intrusion could be as important a determinant as dose in affecting reward potency. When the rewarding dose of drug is injected over a prolonged period of time, the rewarding effects are markedly diminished. Thus, a drug that enters the brain relatively slowly may have diminished rewarding potency as a result. Second, a combination of actions may be required in order to elicit reward. Effects upon nondopamine synapses may either contribute to or diminish reinforcement; since many of the regions thought to be important in the effects of cocaine also receive serotonergic and noradrenergic inputs, it may be necessary to take into account the net effect of any given drug and dose on activity in those regions. For example, although imipramine, like cocaine, is a good inhibitor of serotonin and norepinephrine uptake, it is a relatively poor dopamine uptake inhibitor. This may explain its very weak efficacy as a positive reinforcer.

Although biochemical experiments have given numerous insights into the mechanisms of reinforcement, physiological studies may be required in order to determine the nature of the changes in cellular activity that accompany reward. As was mentioned above, the rate of drug administration is one critical variable in reward; route of administration may be a second, and possibly related, variable. Porrino and Kornetsky (this volume) described 2-deoxyglucose studies demonstrating that the alterations in patterns of cerebral metabolism induced by cocaine are partially dependent upon the route of administration; they are not simply linear functions of dose, but are

affected in complex ways by the amount of drug administered. Given that the patterns of activity revealed by 2-deoxyglucose represent averages of activity over relatively long periods of time, it is clear that the short-term alterations in cerebral function may be even more complex. In this regard, it seems particularly important that more attention be focused on physiological studies of function in defined cell populations. None of the biochemical techniques currently available has a time resolution that even begins to approach that of physiological studies; given the rapid and rate-dependent temporal dynamics of reward, physiological techniques may ultimately be required to characterize the precise nature of the physiological events that accompany reward.

As mentioned previously, a variety of evidence supports the hypothesis that blockade of dopamine uptake is an important aspect of the reinforcement component of the actions of cocaine. However, this implies as a necessary consequence that dopamine receptors are involved in the reinforcing effects of cocaine. The modulation of cocaine reinforcement by dopamine antagonists, as was discussed by a number of speakers, supports this hypothesis. Goeders (this volume) presented evidence that the self-administration of cocaine directly into the medial prefrontal cortex could be blocked by sulpiride, the selective D2 antagonist, but not by a D1 antagonist. Similar issues were addressed by Woolverton and Kleven (this volume), and, like many other aspects of cocaine action, the answers are not simple. In general, dopamine D1 agonists are not self-administered, whereas D2 agonists such as piribedil are. D2 antagonists are effective antagonists of the rewarding effects of cocaine, but there is also some evidence that a D1 antagonist (SCH 23390) can also reduce both the rewarding and discriminative cue properties of cocaine (Woolverton and Kleven, this volume; Koob and Hubner, this volume). The basis for these interactions is obscure but may relate to multiple effects in different brain regions. Alternatively, given the electrophysiological evidence to support the hypothesis that D1 receptors may play a permissive role in the elaboration of dopamine D2 responses (Carlson et al. 1987; White 1987), a similar role for D1 receptors in behavior might hold.

In relation to the role of dopamine in the reinforcing effects of cocaine, Winger (this volume) discussed the possible use of dopamine agonists and antagonists in the treatment of cocaine abuse. Unlike the situation with opiate abusers, where both agonists (e.g., methadone) and antagonists (e.g., naltrexone) can potentially be used as adjuncts to treatment, it seems less likely that either dopamine

agonists such as bromocriptine, or dopamine antagonists such as the neuroleptic drugs, are likely to be as effective in cocaine abusers. Both agonists and antagonists lead to substantial toxicity with chronic use, the agonists producing stereotypic behavior, and the antagonists producing the adverse effects already well established with most neuroleptic drugs, such as tardive dyskinesias, etc. In addition, dopamine antagonists typically increase rather than decrease responding for cocaine injections and decrease responding for other reinforcers such as food; both of these types of effects would clearly be undesirable in a treatment for cocaine abuse. The basis for the rate-increasing effects of dopamine antagonists is still unclear; this has often been interpreted as being a compensatory response to the diminished reinforcing effects of cocaine, but it could also represent an antagonism of the rate-decreasing effects associated with higher doses of cocaine. The relative difficulties that have been encountered in developing practical pharmacological strategies for reducing cocaine self-administration may reflect a more central role for dopamine in reinforcement than for opiates. However, the relative contributions of opiate and dopamine systems in reward, and the relationships between the two systems, are still topics of considerable dispute (Wise and Bozarth 1984; Koob 1987).

Although interactions with dopaminergic systems may be the most important component of the reinforcing effects of cocaine, the relative significance of local anesthetic effects remains unclear. Blood concentrations of cocaine following recreational use reach levels at which local anesthetic actions can be observed, but whether these actions contribute to the rewarding effects of cocaine or represent essentially a "side effect" of this drug is unclear. Local anesthetics commonly induce unique patterns of spindling in olfactory and limbic brain regions, and Post and Weiss (this volume) reported that such effects can be observed in human studies with procaine as well, following intranasal use of cocaine. The precise significance of these effects is unclear, but it was proposed that excessive activity in limbic regions may contribute initially to the euphoric effects of cocaine, but, following pharmacologically induced kindling, may lead to adverse effects such as decreased seizure threshold, panic attacks, etc.

Determining which pharmacological actions of cocaine contribute to specific behavioral effects is clearly a difficult task. A potentially useful tool for approaching these issues was suggested by George and Goldberg (this volume). Studies of cocaine action in different mouse strains have demonstrated markedly different behavioral responses,

particularly in terms of locomotor stimulant effects. By characterizing the physiological and biochemical differences between these strains, it may be possible to dissociate various components of the behavioral response to cocaine and to link them to their appropriate neurobiological substrates. In a similar vein, interspecies comparisons may also be of value in some cases, since some cocainelike drugs (e.g., mazindol) are self-administered in some species but avoided by others.

IDENTIFICATION OF BRAIN REGIONS RELATED TO COCAINE ABUSE

The identification of substrates of drug action within the central nervous system has been a recurring topic of interest in the drug abuse field. Considerable evidence supports a role for the ventral tegmental region and its projection areas in the rewarding effects of not only opiate drugs and psychomotor stimulants, but in the effects of other drugs such as nicotine and alcohol as well. Porrino and Kornetsky (this volume) demonstrated that low doses of cocaine (0.5 mg/kg) could significantly affect glucose utilization in the nucleus accumbens and in the medial prefrontal cortex, both regions that receive important dopamine projections from the ventral tegmental area. At higher doses (e.g., 5 mg/kg), many brain regions showed increases in activity. Whether or not sensitivity of the metabolism of a given brain region to cocaine is necessarily related to reward remains to be determined, but other drugs of abuse that act on dopamine systems, such as amphetamine and methylphenidate, show somewhat similar patterns of activity.

Another approach to identifying important brain sites of cocaine action linked to reward is to combine techniques such as lesions or local administration of drug to defined sites in the brain with behavioral measures of pharmacological reward. In summarizing these types of studies, Koob and Hubner (this volume) presented considerable evidence in support of a central role for the dopamine projections to the nucleus accumbens. Work presented by Koob and Hubner (this volume) and Goeders (this volume) suggested that 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens, but not the frontal cortex, significantly reduced or abolished the reinforcing properties of cocaine. However, in view of the previous observations by Goeders and Smith (1983) that animals will self-administer cocaine into the medial prefrontal cortex, but not the nucleus accumbens, these results are difficult to interpret. Perhaps some of these results could be explained if the mesocortical system were involved in the

initiation aspects of reinforcement, whereas the accumbens is involved in the actual reward process. Thus, lesions of the mesocortical system would only disrupt one of perhaps several initiation systems, whereas accumbens lesions would disrupt the reward system directly. Nevertheless, there remain a number of unanswered questions that can be addressed only through further experiments.

ACUTE TOXICITY

A number of speakers addressed the issue of the toxic effects of cocaine and the hazards associated with its use. Despite the recent occurrence of highly publicized deaths attributed to cocaine, it is clear that, in general, the acute toxicity cannot be particularly high, given the relatively large number of individuals who abuse cocaine. Nevertheless, in specific cases in which individuals have conditions that may predispose them to the toxic effects of cocaine, such as a pseudocholinesterase deficiency or compromised cardiovascular function, acute toxic effects may present a serious risk. Wilkerson (this volume) addressed some of the cardiovascular problems associated with cocaine use, such as myocardial ischemia, ventricular tachycardias, disruptions in local flow, etc., and presented work on a dog model concerning the cardiovascular effects of cocaine. Although the local anesthetic properties of cocaine would be expected to make it an antiarrhythmic drug, both the effects upon the central nervous system (increased sympathetic tone) and the ability of cocaine to potentiate the effects of norepinephrine released at synapses are arrhythmogenic. Determining which of these opposing effects predominate under various conditions and how they relate to the other cardiovascular effects of cocaine will remain an important area of work. Studies on dogs, reported by Wilkerson, clearly stressed the importance of using unanesthetized subjects, since the use of anesthesia blunts the component of the response that is mediated via the central nervous system.

Finally, Dow-Edwards (this volume) described some of the hazards associated with use during pregnancy. Some of the adverse neurological consequences that have been reported in humans may be related to pressor effects of cocaine (e.g., intracephalic hemorrhages), but, in addition, there appear to be more subtle disruptions of development that are manifested only in rather general abnormalities (e.g., "jittery" behavior, decreased interaction, etc.) and remain only poorly understood at this point. Rat studies (Dow-Edwards, this volume)

also have demonstrated lasting changes in both behavior and brain neurochemistry following neonatal cocaine exposure.

EFFECTS OF CHRONIC DRUG USE

The chronic use of cocaine presents two very different and largely unrelated issues. The first concerns changes in sensitivity to the acute effects of cocaine with repeated administration (sensitization), and the second concerns cumulative toxic effects associated with repeated drug administration. While most drugs typically induce a condition of reduced sensitivity upon chronic use (drug tolerance), under certain conditions the repeated use of cocaine can lead to enhanced sensitivity, which was originally described in terms of behavioral sensitization. Repeated use of cocaine can lead to marked potentiation of some of its behavioral effects, such as the increases in motor activity and stereotypy that can be observed following the appropriate dosing regime. Reith (this volume) addressed some of the critical variables in terms of the protocols required to observe behavioral sensitization. In general, these effects are only seen with repetitive administration of lower doses of cocaine, and only with phasic administration of drug. Thus, when the drug was administered chronically with an osmotic minipump, or when high (40 mg/kg) doses were used, locomotor activity following acute cocaine administration seemed to be reduced. However, it remains to be determined whether this reduction in activity constitutes real tolerance or might be the result of the development of "endstage" behaviors (e.g., extreme stereotypy) that might present as reductions in motor activity.

Although the neurochemical bases for sensitization are unclear, some of the biochemical data that were presented by Zahniser et al. (this volume) provided interesting potential directions for research. In these studies, single injections of cocaine were observed to facilitate subsequent amphetamine-induced dopamine release from striatal brain slices for at least 2 weeks. The functional relevance of amphetamine-stimulated release is unclear, but the existence of such long-term changes following treatment with a single dose of cocaine might well provide a mechanism by which sensitization could arise. On the other hand, data presented concerning neurochemical changes following 8 to 14 days of cocaine administration, under conditions that caused behavioral sensitization, did not demonstrate any alterations in dopamine receptors that would suggest potential mechanisms for this process.

With regard to the toxic effects of chronic cocaine administration, several types of studies were presented. Langner et al. (this volume) addressed concerns regarding the potential for cocaine to induce the formation of arteriosclerotic lesions, particularly with repetitive use. Shuster et al. (this volume) described a type of hepatotoxicity that can be observed in mice, particularly following high doses of cocaine. This type of toxicity is considerably more difficult to elicit in rats and may occur relatively rarely in humans, although this is difficult to establish because in many cases the relationship of hepatotoxicity to cocaine abuse may not have been suspected. In mice, a unique but still only partially defined combination of conditions was required to see more severe forms of toxicity (e.g., it did not occur in adrenalectomized mice). Further work may help to identify the conditions under which such toxicity can occur and may provide insights as to which drug abusers are particularly at risk.

Seiden and Kleven (this volume) discussed the neurotoxicity observed with cocaine, amphetamine, and related compounds. Unlike amphetamine, which can release dopamine from the cytosolic pool, drugs that induce release from the granular pool (cocaine, methylphenidate) do not appear to induce neurotoxic changes. A proposed mechanism of action involving the *in vivo* formation of toxic metabolites (e.g., 6-OHDA) was presented, but it will take further work before this can be established.

FUTURE DIRECTIONS

One of the major observations that evolved from this conference was that rapid progress is now being made in characterizing the actions of cocaine on the nervous system, and the behavioral manifestations of such actions. Nonetheless, it is equally clear that the systems upon which cocaine is thought to act are perhaps more complex than would have been suspected based upon the knowledge of these systems that we had even 10 years ago. Despite this complexity, it seems likely that our level of understanding not only of drug abuse per se, but also of the neuronal systems that underlie drug self-administration behavior, should be markedly enhanced by studies now in progress. What is perhaps not as readily apparent is that research on drug abuse is likely to lead to insights not only into drug-seeking and self-administration behaviors, but into a variety of aspects of neurobiology that are only remotely related to drug abuse. In this concluding section, some of these other aspects of this work will be addressed.

There are several levels at which the study of cocaine action upon the central nervous system can prove informative. First, cocaine provides a way in which to perturb neurotransmission at serotonergic, noradrenergic, and dopaminergic synapses. The responses to such changes in terms of receptor binding, transmitter uptake, etc., can tell us much about the complex manner in which such systems are regulated, and about ways that different transmitter systems interact. Many useful antidepressant drugs share pharmacological properties with cocaine, which is not a therapeutically useful antidepressant. By comparing the actions of cocaine to such antidepressant drugs, it may be possible to gain further insights into the specific actions that ameliorate depression, and which changes are unrelated to this therapeutic action. In addition, characterizing the changes that occur following long-term, high-dose use of cocaine, which results in schizophreniclike behaviors in man, may provide a way of understanding some of the biological bases for the schizophrenic process that it emulates.

To a psychobiologist interested in appetitive systems in the brain, cocaine can serve as a remarkably powerful tool that can be employed to define the neuronal circuits involved in pharmacological reward. The observation has frequently been made that associating a specific behavior with cocaine administration is perhaps one of the easiest tasks that a rat can learn. Along these lines, cocaine may ultimately prove to be of interest not only from the standpoint of understanding the neuropharmacological basis for reward but also for providing a key to understanding the learning process itself. Cocaine administration clearly alters the central nervous system in such a manner that events associated with cocaine administration quickly become potent stimuli for eliciting the behaviors that result in drug administration.

In this context, cocaine also provides a ready means of generating positive reinforcement that can be studied with a variety of neurobiological techniques. As one example, cocaine has potential use in defining the physiology of reward, i.e., in determining which patterns of neuronal activity are most closely associated with the behavioral state of positive reinforcement. This in turn may provide a means of understanding some of the more unusual behavioral aspects of reward, such as why a rapid onset of drug action is preferable to a slow onset. In this context, it will also be important to differentiate between various kinds of cocaine administration, such as passive vs. self-administration, and intraperitoneal vs. intravenous administration. By determining how cocaine changes neuronal activity under a variety

of conditions, we may gain insight into how other drugs of abuse act and may ultimately develop more rational methods of treating substance abuse.

From a basic science standpoint, cocaine also provides an invaluable tool for characterizing the high-affinity uptake process for monoamine transmitters. Studies utilizing the binding of radiolabeled cocaine and other inhibitors of uptake have led to the hypothesis that they inhibit uptake by binding to an allosteric regulator of uptake, since dopamine is not an effective competitor for such binding. Further work in this area is likely to lead to a better understanding of the uptake process and its possible regulation by endogenous modulators as well. Studies of other novel aspects of cellular function, such as the calcium-independent release of transmitter from somatodendritic sites in the ventral tegmental area and substantia nigra, have to some extent evolved out of the study of the actions of drugs such as cocaine.

Finally, the study of cocaine might lead to a better understanding of local anesthetic actions in the central nervous system and in the heart. Many of the actions of cocaine remain obscure. Why should a local anesthetic drug cause seizures? Which brain regions are involved? What cellular alterations lead to such excitability, and how can this lead to kindling following repetitive drug administration? In addition, it is unclear how a drug such as cocaine (or other local anesthetics) not only can have antiarrhythmic effects but also can increase the probability of arrhythmias, particularly in lower dose ranges. What we learn from cocaine in this regard will considerably enhance our understanding of the basic biology of these systems.

There clearly remain a number of highly important yet unresolved issues concerning the pharmacological effects of cocaine. In answering earlier questions concerning the actions of cocaine, different and often more complex questions are raised in their stead. However, the results presented in this monograph provide manifest evidence of the rapid evolution of our understanding of the actions of cocaine. The primary intent of this introduction has been to focus attention upon specific areas in which considerable progress has been made in the last few years and, in addition, to suggest areas in which directed efforts might be particularly successful in making further advances. The hope is that a better understanding of the mechanism by which cocaine acts may suggest better ways of treating cocaine abuse. Nevertheless, it is also clear that if a better understanding of the mechanisms underlying drug self-administration can be

achieved, this will necessarily involve significant advances in our understanding of the psychobiological bases for the regulation of appetitive behaviors.

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