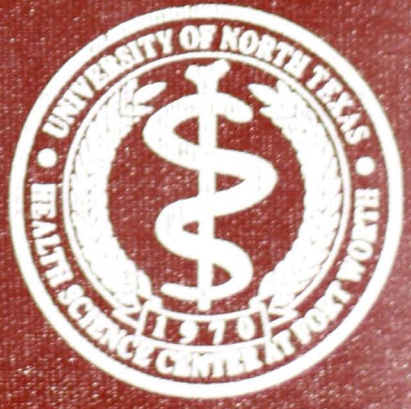
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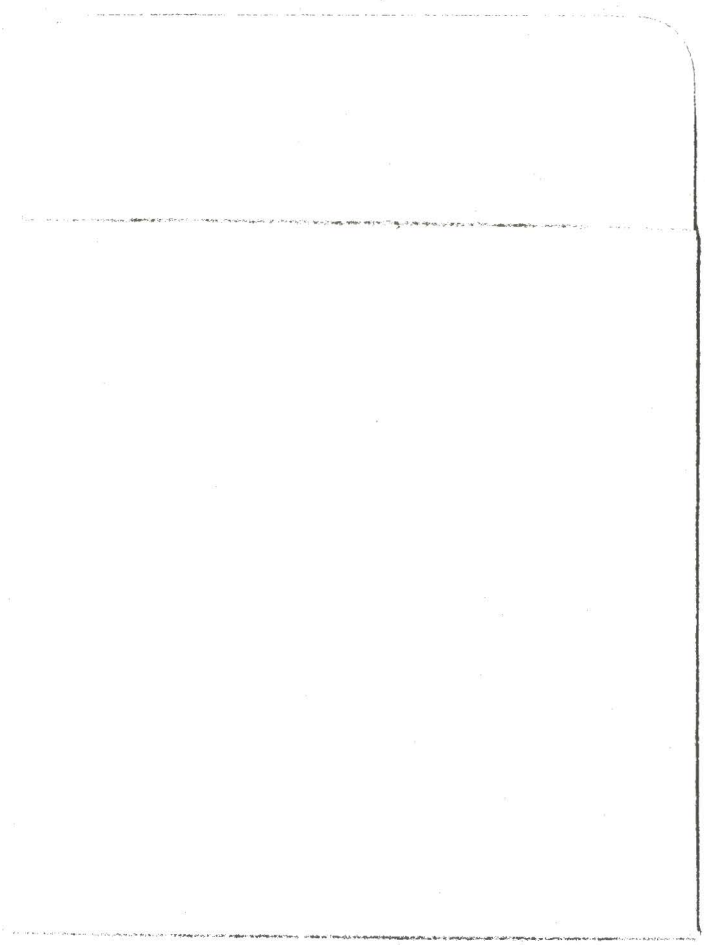

Peltier, Rachel L., The Involvement of D1 and D2 Dopamine Receptors in Cocaine Self-Administration. Doctor of Philosophy (Biomedical Sciences), June 1996, 195 pp. introduction, 6 chapters, discussion, bibliography, 91 titles.

D1 and D2 dopamine receptor subtypes have been implicated in producing the reinforcing properties of cocaine. Chronic exposure to cocaine produces tolerance to its' reinforcing effects in rats trained to self-administer cocaine. The time between cocaine reinforcers (IS^RT) is directly related to dose. A three-point dose-response curve (0.125, 0.25 and 0.5 mg/inj) for cocaine self-administration is obtained during a single test session, allowing determination of optimal tolerance effects of cocaine (20 mg/kg/8 hr/7days; IP) as demonstrated by a shift of the curve to the right. To test if pharmacokinetic factors contribute to the development of tolerance to the reinforcing properties of cocaine (20 mg/kg/8 hr/7days; IP), cocaine and benzoylecgonine (metabolite) were measured in the plasma and brains of rats given a challenge injection of cocaine (2.0 mg/kg; I.V.). Chronic cocaine did not reduce the concentration of cocaine in plasma or brain, indicating that the reduced reinforcing effect of cocaine must be due to pharmacodynamic changes. Acute pretreatment with either the direct dopamine agonist apomorphine (0.32- 3.2 mg/kg), the indirect dopamine agonists *d*-amphetamine (0.32- 3.2 mg/kg) or methamphetamine (1.0

mg/kg) did not consistently change cocaine self-administration. Chronic high-dose treatment with *d*-amphetamine and methamphetamine produced cross-tolerance to the reinforcing effects of cocaine but apomorphine (0.32-3.2 mg/kg) did not. In contrast, acute pretreatment with dopamine antagonists; flupentixol (mixed D1 and D2, 0.032-1.0 mg/kg), SCH23390 (specific D1, 0.0032-0.032 mg/kg), or eticlopride (specific D2, 0.0032- 3.2 mg/kg); dose-dependently decreased the reinforcing effects of cocaine (IS^RT). Chronic treatment with mixed or D1 antagonists (flupentixol ,3.2 mg/kg/12 hr/5 days; or SCH23390, 0.25 mg/kg/12 hr/ 7 days) produced sensitization to the reinforcing effects of cocaine, but the D2 antagonist eticlopride (0.25 mg/kg/12 hr/7 days) produced cross -tolerance to the reinforcing effects of cocaine. In summary, both the D1 and D2 receptor subtypes seem to be involved in the acute effects of cocaine; however, the development of tolerance to cocaine appears to involve only the D1 receptor subtype.

THE INVOLVEMENT OF D1 AND D2 DOPAMINE RECEPTORS IN

COCAINE SELF-ADMINISTRATION

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THE INVOLVEMENT OF D1 AND D2 DOPAMINE RECEPTORS IN COCAINE SELF-ADMINISTRATION

DISSERTATION

Presented to the graduated Council of the

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DOCTOR OF PHILOSOPHY

By

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INTRODUCTION

Psycho-stimulants like cocaine produce euphoria in humans and function as a rewarding stimulus in a variety of species. In addition, humans and animals will both self-administer cocaine (Johanson and Fischman, 1989). It has been postulated that it is the rewarding effects of cocaine and other drugs of abuse which are responsible for their abuse liability. What is not in question; however, is that cocaine abuse continues to be a significant problem in the United States. Behavioral pharmacologists are attempting to alleviate this problem by developing pharmacological treatments that would reduce cocaine abuse and/or augment current treatment programs in preventing relapse. In order to produce an effective pharmacotherapeutic treatment for cocaine abusers, we must begin by first understanding what neurochemical systems are actually involved in mediating the reinforcing effects of cocaine.

The reinforcing properties of drugs of abuse, including cocaine, derive from their ability to change the activity of neurotransmitter systems utilized by natural reinforcers such as food, water, sex, etc. (Di Chiara and North, 1992; Koob 1992a). Among the neural pathways that are linked to

motivation, the one involving dopamine (DA) appears to be the most critically involved in the action of drugs of abuse (Di Chiara, 1995).

Dopamine is a neuromodulator that is unable, by itself, to either trigger or inhibit the generation of action potentials by *directly* operating ion channels and thus either depolarizing or hyperpolarizing the neuronal membrane. DA receptors, instead, alter the responsiveness of neuronal membranes to fast neurotransmission by modifying the voltage dependence of voltage-operated ion channels (Kitai and Surmeier, 1993; Di Chiara *et al.*, 1994). Dopamine receptors can be divided into two major subtypes, D1- and D2-like receptors. Direct stimulation of D1 receptors produces a shift towards lower membrane voltage of the voltage sensitivity of the K^+-channels, making the neuron less resistant to burst-firing stimulation by fast excitatory transmission; while stimulation of D2 receptors is thought to influence K^+ currents in a manner just opposite to that of D1 receptors, making the neuron more resistant to burst-firing stimulation by fast excitatory transmission (Kitai and Surmeier, 1993). In addition to its actions on post-synaptic DA receptors, DA can also act on pre-synaptic DA receptors, mainly of the D2 type, to inhibit its own release.

Drugs of abuse have been shown to increase extracellular DA in various terminal DA areas as estimated by brain microdialysis in freely-moving rats (DI Chiara and Imperato, 1988a). More specifically, *cocaine*

increases extracellular DA by blocking the reuptake of exocytotically released DA, which is both calcium- and impulse-dependent (Carboni *et al.*, 1989). It has been demonstrated, using brain microdialysis, that cocaine increases DA to a much larger extent in the nucleus accumbens than in either the dorso-lateral caudate-putamen (Kuczenski and Segal, 1992) or the prefrontal cortex (Moghaddam and Bunney, 1989).

Although cocaine blocks DA, norepinephrine (NE) and Serotonin (5-HT) reuptake as well as having local anesthetic properties, indirect evidence suggests that it is self-administered because of its DA stimulant properties that are related directly to its ability to block DA reuptake (Di Chiara, 1995). Thus, within a series of cocaine congeners the ability to substitute for cocaine self-administration was directly related to their ability to bind to the reuptake carrier on the cocaine recognition site (Ritz *et al.*, 1987). More specifically, GBR 12909, a specific DA reuptake inhibitor, is fully active as a cocaine substitute (Bergman *et al.*, 1989; Howell and Bird, 1991; Roberts, 1993) while selective NE and 5-HT reuptake blockers are inactive (Woolverton, 1987).

Further evidence for a role of DA in the reinforcing properties of cocaine has been provided by examining the effects of 6-hydroxydopamine (6-OHDA) lesions on cocaine self-administration. Thus, 6-OHDA lesions of the DA innervation in the nucleus accumbens results in extinction-like ef-

fects on responding for cocaine in fixed ratio (FR) schedules (Roberts *et al.*, 1977; Roberts *et al.*, 1980; Pettit *et al.*, 1984) and in a decrease in breaking-point in progressive ratio (PR) schedules (Koob *et al.*, 1987). In both cases, these results indicate that intact DA innervation in the nucleus accumbens is required for cocaine reinforcement.

In summary, although the above evidence provides evidence for a strong relationship between the property of stimulating DA transmission and the abuse liability of cocaine, the exact role played by DA receptors is unclear. It would stand to reason; therefore, that the most logical place to begin an investigation of dopamine's role in cocaine reinforcement would be to determine the involvement of the D1 and D2 dopamine receptor subtypes. The working hypothesis for the following set of experiments is: *The reinforcing effects of cocaine require the activation of dopamine receptors (D1 and/or D2).*

In designing the following experiments, I have chosen to use a cocaine self-administration paradigm for the behavioral measures. Self-administration is an operant behavior that is maintained by response-contingent drug-administration. I chose this method specifically because self-administration in animals is currently considered the most reliable measure of the abuse potential of drugs because it possesses not only face but also predictive and construct validity as a model of human drug abuse

(Yanagita, 1977; Pickens *et al.*, 1978; Johanson and Schuster, 1981; Markou *et al.*, 1993). Given this, results obtained using this paradigm will provide important data on the role of DA in the reinforcing properties of cocaine abuse and in the mechanism of drug abuse.

The following portion of this introduction will consist of a Background and Significance section for each of the experiments that were conducted. It is divided into separate sections, each corresponding to a specific set of experiments, in order to make it easier for the reader to follow the logical sequence of events.

Single-Dose / Multi-Day vs. Multi-Dose / Single-Day Test Procedures

The main goal of this set of experiments was to develop an improved method for determining a dose-response curve for cocaine self-administration in a shorter amount of time. The need for a quick dose-response determination arose specifically in this lab when it was determined that tolerance produced by chronic cocaine lasts for a short amount of time, during which a dose-response curve for cocaine self-administration needed to be determined. Therefore, the specific aims for these experiments were: 1. *To determine if a multi-dose test procedure produces dose-response curves for cocaine self-administration that have similar Inter-reinforcer times (IS^RT's) as dose-response curves obtained using a single-*

dose test procedure. 2. To determine if a multi-dose test procedure can be used to measure a maximal shift in the dose-response curve for cocaine self-administration produced by chronic cocaine treatment.

Tolerance develops to the DA reuptake blocker, cocaine, in both drug discrimination (Barrett and Leith, 1981; Wood and Emmett-Oglesby, 1986, 1987, 1988, 1989; Young and Sannerud, 1989; Steigerwald *et al.*, 1994) and drug self-administration paradigms in rats (McCown and Barrett, 1980; Emmett-Oglesby and Lane; 1992; Emmett-Oglesby *et al.*, 1993; Li *et al.*, 1994). Tolerance to the reinforcing effects of cocaine has also been demonstrated in rats trained to self-administer cocaine under both low value fixed ratio (FR) (Emmett-Oglesby and Lane; 1992; Emmett-Oglesby *et al.*; 1993) and progressive ratio (PR) schedules (Li *et al.*, 1994). Rats trained to self-administer 15 injections of 1.0 mg/kg/inj of cocaine under an FR2 schedule of reinforcement demonstrated a two-fold shift to the right of the cocaine self-administration dose-response curve after receiving intravenous cocaine (20 mg/kg/8 hr for 7 days) (Emmett-Oglesby and Lane; 1992). With this regimen, a maximal degree of tolerance develops within 4 days, and when the chronic regimen is discontinued, this effect disappears in approximately 2-4 days (Emmett-Oglesby and Lane, 1992). In that study, dose-response data were assessed during the four days following termination of chronic dosing. Self-administration dose-effect testing was per-

formed using *a single-dose* test procedure instead of a *dose-response* determination (i.e. a different dose of cocaine was tested on each of these 4 days). Although this procedure allows one to examine the effects of chronic cocaine, the shape of the dose response curve is changed by the disappearance of tolerance over the test period. Thus, the single-dose testing method does not ensure determination of effects of chronic dosing regimens during an optimally effective time period. In addition, this method may not be effective in the proposed experiments because there is no current information describing the time course of the chronic effects of these compounds. Because, the single-dose testing method does not ensure determination of effects of chronic dosing regimens during an optimally effective time period, it would be beneficial to document tolerance more readily by collecting dose-response data during a single day.

This potential problem has been circumvented in primate self-administration experiments (Winger *et al.*, 1989). Winger and colleges (1989) have designed a method for examining an entire self-administration dose-response curve in a single test session. In that study, they changed the dose of cocaine that was self-administered in a single test session by changing the infusion amount (infusion time) of the IV. delivered reinforcer, while the concentration of cocaine remained constant. This method was very effective and the results were comparable to those obtained using a

single-dose testing method. This problem is more complicated in rats because of their smaller body size. It is possible that because of their size, changing the amount of fluid that is delivered might affect the rate of absorption and/or distribution. I have attempted to solve this problem in rats by changing the concentration of cocaine that is delivered and leaving the infusion volume the same.

I have also examined whether this multi-dose procedure is equivalent to the single-dose procedure by a direct comparison of the two methods in the same group of rats. In addition, I have determined the reproducibility of the multi-dose method by using this procedure to repeatedly test the same group of rats. The last test for this method was to determine whether it would allow us to examine the effects of chronic cocaine treatment on the dose-response curve for cocaine self-administration. In other words, would it allow us to measure tolerance to the reinforcing effects of cocaine during the period of time during which the dose-response curve for cocaine self-administration is maximally shifted.

Pharmacokinetic vs. Pharmacodynamic Tolerance

The main goal of this set of experiments was to determine whether tolerance to the reinforcing effects of cocaine produced by chronic cocaine treatment was due to pharmacokinetic factors. Therefore, the specific aims

for this set of experiments were: 1. *To determine if the IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) will result in a similar degree of tolerance as produced by the IV administration of cocaine.* 2. *To determine if IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) produces pharmacokinetic tolerance to the reinforcing effects of cocaine as measured by plasma and brain levels of cocaine and benzoylecgonine (BZE).*

Rats trained to self-administer cocaine under an FR2 schedule of reinforcement demonstrated an orderly increase in the time between injections as the dose of cocaine is increased. In other words, as the dose of cocaine increases, the rate of self-administration decreases. Cocaine given chronically produces tolerance by shifting the dose-response curve for cocaine self-administration to the right or down (Emmett-Oglesby and Lane, 1992; Depoortere *et al.*, 1993). What is not known is whether this chronic cocaine regimen is producing pharmacodynamic or pharmacokinetic tolerance. Either scenario could explain why the chronic cocaine regimen would result in cocaine being self-administered at a higher rate. For example, if chronic cocaine is producing pharmacodynamic tolerance it would reduce the reinforcing properties of cocaine by decreasing the number of active receptors in the brain. If this is the case, then a given dose of cocaine would be perceived as a lower dose after the chronic regimen. Chronic cocaine could also be producing pharmacokinetic tolerance, the

end result of which would be a decrease in the amount of drug that is actually available in the brain to interact with the receptors.

There are several ways that pharmacokinetic tolerance could be produced. For example, chronic cocaine treatment could increase the rate at which cocaine is metabolized. To date, we have never experimentally examined the effects of cocaine (20 mg/kg/8 hr/7 days) on cocaine metabolism. Therefore, in these experiments, I have examined the effects of chronic cocaine (20 mg/kg/8 hr/7 days; IP) on cocaine metabolism by first measuring plasma concentrations of cocaine and benzoylecgonine both before and after chronic cocaine treatment using a gas chromatographic / mass spectrometry (GCMS) analysis. If chronic cocaine increases the rate at which cocaine is being metabolized, I would expect to see a lower concentration of cocaine and a higher concentration of benzoylecgonine in the plasma. If this is the case, it could explain the behavioral data in the following way: a given dose of cocaine would now be self-administered at a faster rate because there would actually be a lower concentration of cocaine interacting with the receptor.

The second measure that I will use to determine if chronic cocaine (20 mg/kg/8 hr/7 days; IP) changes the amount of cocaine available to interact with receptors, is to measure the concentration of cocaine present in the brain after chronic treatment with either cocaine or saline again using a

GCMS analysis. If chronic cocaine increases cocaine metabolism, I would expect to see a decrease in the concentration of cocaine in the brain.

The previous experiment that demonstrated tolerance to the reinforcing effects of cocaine examined the intravenous (IV) administration of chronic cocaine (Emmett-Oglesby *et al.*, 1993). In that study, rats lived in the operant chambers during the chronic treatment regimen, and the cocaine was delivered IV.

For the proposed pharmacokinetic experiments, it is important that the jugular catheters have a limited exposure to cocaine. The reason for this caution, is that it is possible that the silastic polymer with which the catheters are constructed may leach cocaine if they are in contact with cocaine-containing solutions for extended periods of time (as is the case when chronic cocaine is delivered by the IV route). Therefore, the first experiment will be conducted to determine if the Intraperitoneal (IP) administration of chronic cocaine will produce comparable tolerance to the reinforcing effects of cocaine as does chronic cocaine administered by the IV route.

Acute Treatment with DA agonists and antagonists

The main goal of these experiments was to determine the effects of **acute** treatment with: direct and indirect DA **agonists**, D1 and D2 dopamine **antagonists** on cocaine self-administration in rats. The specific aims for this set of experiments were: 1. *To determine if the acute administration of either direct or indirect DA agonists will augment the reinforcing effects of cocaine*; 2. *To determine if the acute administration of direct DA antagonists block the reinforcing effects of cocaine.*

As I mentioned earlier, the reinforcing effects of cocaine have been examined and appear to be mediated, at least in part, by the mesocorticolimbic dopamine system (Goeders and Smith, 1983, 1986; Post *et al.*, 1987; Roberts and Koob, 1982; Roberts *et al.*, 1977, 1980). A unique property of drug self-administration, is that animals maintain a relatively stable level of drug intake over time (Pickens *et al.*, 1978; Yokel and Pickens, 1974). For example, animals respond to changes in the injection dose by increasing their rate of self-administration following decreases in dose and decreasing their rate of self-administration following increases in the injection dose (i.e. there is an inverse relationship between dose and rate of self-administration)(Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Goldberg *et al.*, 1971; Pickens and Thompson, 1968; Wilson *et al*, 1971; Woods and Schuster, 1968).

With the exception of the work published by Emmett-Oglesby *et al.* (1993), the other reports measured the direct effects of dose changes on the rate of self-administration (Caine and Koob, 1994; Goldberg *et al.*, 1971; Pickens and Thompson, 1968; Wilson *et al*, 1971; Woods and Schuster, 1968). The experimental sessions were limited by time; however, the subjects in these experiments were allowed to self-administer an unlimited amount of drug. The data obtained from this type of paradigm is measured as the number of reinforcers per hour. With this measure, however, there is a large amount of *intra*-session, *inter*-session and inter-animal variability. In addition, this variability actually increases as you increase the session time.

As I mentioned above, our laboratory does not directly measure the rate of cocaine self-administration in the manner described above (Emmett-Oglesby *et al.*, 1993) Instead of limiting the test/training sessions by time, we limit the number of cocaine injections that are available for self-administration. Our training sessions consist of 15 reinforcers (0.25 mg/injection of cocaine) and an unlimited amount of time in which to obtain them. Similarly, our test sessions consist of 24 reinforcers (8 reinforcers of 0.5 mg/injection; 8 reinforcers of 0.25 mg/injection; and 8 reinforcers of 0.125 mg/injection) and an unlimited amount of time in which to obtain them. We chose to limit the number of reinforcers instead of limiting time

for two reasons: 1) we primarily investigate the development of tolerance and/or cross-tolerance to the reinforcing effects of cocaine, and we wanted to reduce the chance that tolerance would develop during the training sessions; 2) Limiting the number of available reinforcers decreases the variability. The data that we obtain is represented as the average time occurring between each reinforcer, or IS^RT, in minutes. This is an indirect measure of rate; therefore, in our hands, a decrease in the unit dose of cocaine is represented as a decrease in the IS^RT; and conversely, an increase in the unit dose of cocaine is represented as an increase in the IS^RT (i.e. there in a direct relationship between dose and IS^RT).

Dopamine antagonists are thought to block the reinforcing effects of cocaine, because increased rates of cocaine self-administration are produced by pretreatment with these compounds (Britton *et al.,* 1991; Caine and Koob, 1994; Ettinberg *et al.*, 1982; Roberts and Vickers, 1984); or, in this laboratory, decreased IS^RTs (Emmett-Oglesby *et al.*, 1993). Again, because of the difference in the measures, both of these findings (increased rates of cocaine self-administration and decreased IS^RTs) are indicative of a decrease in dose; hence, a blockade of the reinforcing effects of cocaine.

In contrast to blockade of the reinforcing effects of cocaine by dopamine antagonists, acute pretreatment with the indirect *dopamine agonist* *d*-amphetamine results in a decrease in the rate of either cocaine or *d*-

amphetamine self-administration (Pickens *et al.*, 1968; Wilson and Schuster, 1973).

As compared to the volumes of literature describing the effects of dopamine *antagonists* on cocaine self-administration, there are very few reports in the literature describing the effects of dopamine *agonists*. The work that has been performed; however, have only examined the effects of *indirect* dopamine agonists. Therefore, the present experiment was designed to replicate and extend these results by examining the effects of direct (**apomorphine**) and indirect (***d*-amphetamine** and **methamphetamine**) dopamine agonists on cocaine self-administration. In addition, these experiments will also compare the effects of the mixed dopamine antagonist **flupenthixol** with the specific D1 antagonist **SCH23390** and the specific D2 antagonist **eticlopride** on cocaine self-administration.

Chronic Treatment with DA Agonists

The effects of acute treatment with dopaminergic agonists provides us with information concerning the role of DA in cocaine reinforcement; however, actual treatment regimens in human drug abusers would require that the patient be treated chronically. Therefore, it is important that we know what effects the chronic treatment with these drugs have on cocaine self-administration. The specific aim for this experiments was: *To deter-*

mine if the chronic administration of either direct or indirect DA agonists will result in cross-tolerance to the reinforcing effects of cocaine.

Although there are many reports in the literature describing the *acute* effects of DA agonist and antagonist on cocaine self-administration, there have been very few experiments that have examined the *chronic* effects of these same compounds. In theory, the chronic administration of an "agonist" should result in a decrease in functional receptors, producing a decreased effectiveness of that drug, or *tolerance*. Similarly, the chronic treatment with "other agonists" that have a similar mechanism of action, should also result in a decrease in functional receptors, producing a decreased effectiveness of both drugs, or *cross-tolerance*. In our laboratory, tolerance should be observed by a shift to the right and/or down of the dose-response curve for cocaine self-administration. In other words, if tolerance means that a set dose of cocaine now has the reinforcing effects of a lower dose, there should be a decrease in the IS^RT for that dose.

As I stated earlier, tolerance has been shown to develop to CNS stimulants in both drug discrimination (Barrett and Leith, 1981; Steigerwald *et al.*, 1994; Wood and Emmett-Oglesby, 1986, 1987, 1988, 1989; Young and Sannerud, 1989) and drug self-administration paradigms in rats (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.,* 1993; Li et al., 1994; McCown and Barrett, 1980). For example, Wood and Emmett-

Oglesby (1986, 1988) showed that in rats trained to discriminate cocaine, 10 mg/kg, from saline, a two-fold shift to the right of the cocaine dose-effect curve occurred after 7 days of chronic treatment with cocaine, either 10 or 20 mg/kg/8 hr. Using *d*-A as a training drug, Barrett and Leith (1981) showed that rats treated chronically with a total of 78 mg/kg of *d*-amphetamine over three days were tolerant to the training dose of *d*-amphetamine. Similarly, Steigerwald *et al.* (1994) showed that rats trained to discriminate *d*-amphetamine, 0.80 mg/kg, from saline demonstrated a three-fold shift to the right of the *d*-amphetamine dose-effect curve after chronic treatment with *d*-amphetamine, 3.2 mg/kg/12 hr for 7 days, and this tolerance increased to a four-fold shift after 14 days of treatment.

This laboratory has also demonstrated tolerance to the reinforcing effects of cocaine in rats trained to self-administer cocaine under both low value FR (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993) and PR schedules (Li *et al.,* 1994). In parallel to the data obtained in the cocaine discrimination studies, rats trained to self-administer 15 injections of 1.0 mg/kg/inj of cocaine under an FR2 schedule of reinforcement demonstrated an approximate two-fold shift to the right of the cocaine self-administration dose-response curve after receiving intravenous cocaine (20 mg/kg/8 hr for 7 days) (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.,* 1993). Similarly, rats trained to self-administer cocaine (0.9

mg/kg/injection) under a PR schedule, where an increasing number of responses is required to complete the ratio for each subsequent reinforcer, showed approximately two-fold tolerance to the reinforcing effects of cocaine after receiving intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days; Li *et al.*, 1994). In addition, tolerance to the reinforcing effects of *d*-amphetamine has also been demonstrated in rats trained to self-administer *d*-amphetamine under an FR1 schedule of reinforcement (McCown and Barrett, 1980). In that study, rats were trained to self-administer *d*-amphetamine, either 0.125 or 0.25 mg/kg/inj. Following chronic treatment with 78 mg/kg of *d*-amphetamine over three days, all subjects showed an increase in the amount of *d*-amphetamine self-administered by at least 45% over baseline levels.

So far, there has been no examination of the cross-tolerance profiles of CNS stimulants in a self-administration paradigm. Based on the drug discrimination data obtained in this laboratory, I have selected two well characterized CNS stimulants of the amphetamine type (*d*-amphetamine and methamphetamine) to examine for their potential to produce cross-tolerance to the reinforcing effects of cocaine. I will also examine the ability of a *direct* dopamine agonist to produce cross-tolerance or cross-sensitization to the reinforcing effects of cocaine. For this experiment I have chosen to test the D1/D2 agonist, apomorphine.

Chronic Treatment with DA Antagonists

Again, it is critical for the evaluation of these compounds as potential pharmacotherapeutic treatments that we know how chronic treatment with these drugs effects cocaine self-administration. Therefore, the specific aim for this experiments was: *To determine if the chronic administration of direct DA antagonists will result in cross-sensitization to the reinforcing effects of cocaine.*

Although there is a substantial database describing the effects of dopamine antagonists on cocaine self-administration, the majority of these reports examine the acute effects of these compounds. In fact, only a limited number of reports describe chronic treatment effects of dopamine antagonists (Richardson *et al.*, 1994; Roberts and Vickers, 1987). Chronic haloperidol injections (0.075 mg/kg/day for 1 week; IP) produce a progressive increase in cocaine self-administration (Roberts and Vickers, 1987). Similarly, one IM injection of the deconate form of flupenthixol (2.0 mg) or haloperidol (2.5 mg) produces a progressive increase in cocaine self-administration in a low value fixed-ratio paradigm (Richardson *et al.*, 1994). Both haloperidol and flupenthixol are mixed D1/D2 antagonists; therefore, these reports do not demonstrate the contribution of the individual receptor subtypes.

In theory, the chronic administration of an "antagonist" should result in an increase in functional receptors, producing an increased effectiveness of that drug, or *sensitization.* Similarly, the chronic treatment with "other antagonists" that have a similar mechanism of action, should also result in an increase in functional receptors, producing an increased effectiveness of both drugs, or *cross-sensitization.* In our laboratory, sensitization should be observed by a shift to the left and/or down of the dose-response curve for cocaine self-administration. In other words, if tolerance means that a set dose of cocaine now has the reinforcing effects of a higher dose, there should be an increase in the IS^RT for that dose.

The aim of the present experiment is to determine the effects of chronically administered dopamine antagonists on cocaine self-administration, starting with the nonspecific dopamine antagonist flupenthixol. In addition we will determine the individual involvement of: 1) the D1 receptor subtype by examining the effects of chronic treatment with SCH23390; 2) and the D2 receptor subtype by examining the effects of chronic treatment with eticlopride.

A summary for the specific aims for this project are as follows:

1. To determine if a multi-dose test procedure produces dose-response curves for cocaine self-administration that have similar Inter-

reinforcer times (IS^RT's) as dose-response curves obtained using a single-dose test procedure.

2. To determine if a multi-dose / single day test procedure can be used to measure a maximal shift in the dose-response curve for cocaine self-administration produced by chronic cocaine treatment.

3. To determine if the IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) will result in a similar degree of tolerance as produced by the IV administration of cocaine.

4. To determine if IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) produces pharmacokinetic tolerance to the reinforcing effects of cocaine as measured by plasma and brain levels of cocaine and benzoylecgonine (BZE).

5. To determine if the acute administration of either direct or indirect DA agonists will augment the reinforcing effects of cocaine.

6. To determine if the acute administration of direct DA antagonists block the reinforcing effects of cocaine.

7. To determine if the chronic administration of either direct or indirect DA agonists will result in cross-tolerance to the reinforcing effects of cocaine.

8. To determine if the chronic administration of direct DA antagonists will result in cross-sensitization to the reinforcing effects of cocaine.

CHAPTER 1

The experiments in this chapter were designed to test the following specific aims: 1) To determine if a *multi-dose test procedure* produces dose-response curves for cocaine self-administration that have similar Inter-reinforcer times (IS^RT's) as dose-response curves obtained using a *single-dose test procedure*; 2) To determine if a multi-dose / single day test procedure can be used to measure a maximal shift in the dose-response curve for cocaine self-administration produced by chronic cocaine treatment. The development of a multi-dose method will allow more precise characterization of the development and recovery from cocaine tolerance. This manuscript is published in *Drug. Alcohol. Dep.* 32:247-256, 1993.

Tolerance to self-administration of cocaine in rats: Time course and dose-response determination using a multi-dose method[1]

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ABSTRACT

To assess tolerance to cocaine in a self-administration paradigm, rats were trained to self-administer cocaine (0.25 mg/injection) on a fixed-ratio 2 (FR2) schedule of reinforcement. The development of tolerance was studied during chronic administration of cocaine (20 mg/kg per 8 h for 10 days), given either contingently (self-administered by the rats) or non-contingently (infused by the experimenter). Both contingent and non-contingent administration of cocaine produced comparable tolerance, as indicated by a faster rate of cocaine self-administration (the average inter-reinforcer time, IS^RT, decreased significantly). Tolerance developed by day 2 of the chronic regimen and reached a floor value (60% of baseline) from day 4 through day 10. Termination of chronic cocaine then resulted in recovery from tolerance, with IS^RT's returning to baseline within 6 days or termination. A second set of experiments determined whether tolerance could be studied using a multi-dose method to obtain dose-response data in a single session. A system of multiple pumps allowed testing of three doses of cocaine during a single experimental session. Cocaine dose-response curves obtained from the multi-dose method: (I) did not differ from that obtained from a single-dose method; (ii) were reproducible; and (iii) were shifted to the right by Scherring 23390. Rats were then subjected to a 7-day chronic regimen of infused cocaine (20 mg/kg per 8 h) or infused saline. At the end of this chronic cocaine period, they were then tested with the multi-dose method. Chronic cocaine, as compared to chronic saline, shifted the cocaine dose-

response curve to the right, indicating that the multi-dose method can be successful applied to demonstrate tolerance to the effects of cocaine in a self-administration paradigm.

Key Words: cocaine; tolerance; self-administration; multi-dose; rat

INTRODUCTION

We recently reported (Emmett-Oglesby and Lane, 1992) that tolerance occurs to the reinforcing effects of cocaine. In that experiment, rats were trained to self-administer cocaine, and tolerance was then produced by suspending training and infusing cocaine, 20 mg/kg per 8 h for a week. This chronic regimen of cocaine caused a rate of self-administration that was nearly twice as fast as that seen before chronic treatment. The study by Emmett-Oglesby and Lane (1992) was modeled on findings from the drug discrimination literature, where tolerance to the discriminative stimulus effects of cocaine has been well documented (McKenna and Ho, 1977; Wood and Emmett-Oglesby, 1986, 1987, 1988, 1989). Using the dosing regimen of 20 mg/kg per 8 h, tolerance to the discriminative stimulus effects of cocaine has been shown to have an orderly onset and, upon termination of chronic treatment, an orderly offset (Wood et al., 1984; Wood and Emmett-Oglesby, 1986). The onset did not occur until at least

2 days of chronic treatment had elapsed (Wood *et al.*, 1984), and the offset required even longer following termination of treatment. In the present study we determined the rate of onset, the duration and the rate of offset of tolerance to self administered cocaine by exposing subjects to a 10-day regimen of cocaine 20 mg/kg per 8 h.

The magnitude of tolerance to drug effects has been shown to be dependent upon factors such as contingency between drug administration and behavioral testing (Wolgin, 1989; Siegel, 1989). In addition, response-contingent versus response-non-contingent delivery of drug has been shown to result in differential neurochemical changes (e.g., Smith *et al.* , 1980). Therefore, we also determined whether a significantly different degree of tolerance was produced when cocaine was delivered noncontingently (infused by the experimenter) versus contingently (self-administered by the subject).

In the first demonstration of tolerance to the reinforcing effects of cocaine (Emmett-Oglesby and Lane, 1992), dose-response data were assessed during four days following termination of chronic dosing. A different dose of cocaine was tested on each of these 4 days. Because of the possibility that tolerance may wax or wane during period, it would be beneficial to document tolerance more readily by collecting dose-response data during a single day. Using primates as subjects, Winger *et al.* (1989) have described a method for determining, in a single session, the dose-response curve from a self-administration ex-

periment. The dose of cocaine infected was varied by changing the volume of drug solution, which was achieved by varying the duration of activation of a single pump. If this method could be adapted to studies of tolerance, it would by possible to determine dose-response functions during a single session, and thus waxing or waning of tolerance over days would not be a factor. In the present experiments, we have modified the Winger *et al.* (199) procedure: rather than changing the volume delivered of a single concentration of cocaine, different doses of cocaine were given by using separate syringes that contained different concentrations of the drug. This method provides a constant volume of injection, which results in the same rate of delivery for all doses.

In an initial experiment, we compared data obtained from this multi-dose method to data obtained form a single-dose method; we subsequently tested the reproducibility of data obtained from the multi-dose method by determining the dose-response curve on three occasions; finally, we examined whether Schering 23390 (SCH23390), which is known to block the reinforcing effects of cocaine in rats tested by a single-dose method (Koob *et al.* 1987; Hubner and Moreton, 1991; Lane *et al.* 1992), would also shift the dose-response curve for cocaine obtained from this multi-dose method. When these tests were completed, we determined whether this method could be used to demonstrate a shift to the right of the cocaine dose-response curve following chronic administration of cocaine.

METHODS

Subjects. Thirty-five male Fisher F-344 rats were used as subjects. They were maintained at 250 ± 10g by restricting their access to food. The subjects were housed singly and kept on a 12-h light/dark cycle with lights on a 7:00 am.

Apparatus. Lever-press shaping and self-administration experiments took place in custom-designed and locally built operant chambers that were enclosed in light- and sound-attenuating enclosures. All experimental contingencies were controlled and data were collected via MS-DOS compatible microcomputers using software described previously (Emmett-Oglesby *et al.*, 1982). The chambers contained a single lever, which could be depressed by a force of 0.15 N, and which was mounted on the back wall 5 cm above the floor. Chambers were equipped with two stimulus lights (3 W), one located on the ceiling and the other 9 cm above the lever. Syringe pumps (Razel, Model A; driven at 3.33 RPM) were used to drive 10-ml plastic syringes located outside of the enclosures. Four sets of pump/syringe were used for each self-administration chamber: three syringes contained cocaine solution (0.125, 0.25 or 0.5 mg/0.1 ml injection), and the fourth syringe contained a saline-vehicle solution (0.9% saline with 4 units heparin/ml). The four syringes were connected through a series of Tygon tubing (0.06 inch O.D. Tygon Microbore Tubing, Norton Performance Plastics, Akron OH) and micro-volume T-connectors (Model N-06365-70, Cole-Parmer, Chicago,

IL) to a single-channel fluid swivel (Model 375/22, Instech Laboratories, Inc., Plymouth Meeting, PA). A Tygon line, enclosed in a steel spring, exited the swivel and immediately entered the chamber via a 1.0-cm hole in the center of the chamber ceiling. The swivel and line were mounted on a counter-balanced arm (for additional details see Emmett-Oglesby and Lane, 1992). The total dead space from the T-connectors to the tip of the catheter was approximately 0.12 ml. Operant chambers used for the chronic experiments (see Experiment 1 and 3) were equipped with a retractable lever (Model E23-07, Coulbourn Instruments, Allentown, PA) and a water bottle.

Lever-press shaping. Rats were first trained to press a lever on a fixed-ratio on schedule (FR1) using food (45 mg pellets) as a reinforcer. After lever-pressing was acquired, the schedule of reinforcement was increased to FR2, with the delivery of each reinforcer followed by a 30-s time-out period in the dark. Pressing the lever during this time-out period had no consequences. Subjects were implanted with catheters when a least 70% of their lever-presses were emitted when the stimulus lights were on.

Cocaine self-administration training. Details of surgical procedure and assessment of catheter patency before each daily self-administration session have been described previously (Emmett-Oglesby and Lane, 1992). Five days

after catheter implantation, subjects were trained to self-administer cocaine everyday. At the beginning of each session, a priming injection of 0.3 mg of cocaine was given. Subjects were first trained on a FR1 schedule with a maximum of 25 cocaine injections. Each injection (0.25 mg of cocaine/0.1 ml) was accompanied by flashing of the stimulus lights and was followed by a 30-s time-out period in the dark. Pressing the lever during this time-out period had no consequences. Once rats self-administered all 25 injections within 3 h for two consecutive training sessions, they were switched to a FR2 schedule. From this point forward, training sessions consisted of 15 reinforcers and were limited to 3 h . Rats were tested (see below) once the criterion of stability was met. This criterion consisted in the inter-reinforcer time (IS^RT, in min) not varying by more than 20% across three consecutive training sessions.

Experiment 1: Acquisition and loss of tolerance to cocaine. Subjects were placed in operant chambers equipped for chronic experiments for a period of 10 days. Levers were retracted except when cocaine was available for self-administration. A 12-h light/dark cycle with lights on at 07:00 h was maintained. The rats were randomly assigned to one of two groups. One group (infused; n = 9) was infused with cocaine (20 injections of 0.25 mg/0.1 ml, spaced one min apart) at 08:00 h, 16:00 and 12:00 h. To assess the rate of development of tolerance, the 08:00 h infusion session was replaced by a self-administration ses-

sion every second day. The other group (self-Administered; n = 5) was allowed to self-administer cocaine at 08:00 h , 16:00 h and 12:00 h. For both groups, the twentieth injection of cocaine (infused or self-administered) was followed by a 0.2 ml saline-vehicle flush, and this flush was repeated every 2 h until the start of the next session. After this 10-day period of chronic cocaine was terminated, the rate of offset of tolerance was assessed by allowing rats from both groups to self-administer cocaine every second administration sessions consisted of 20 injections of cocaine, 0.25 mg/0.1 ml, with no time limit to finish the session.

Experiment 2 : Multi-dose method: comparison with single-dose method, reproducibility, and effect of pretreatment with SCH 23390. In the first part of this experiment, cocaine dose-response curves were determined in 10 rats using successively single-dose and multi-dose testing methods. In the single-dose method, 3 doses of cocaine (0.125, 0.25, 0.5 mg/injection for 15 injections) were tested in a random order, 1 dose/day. In the multi-dose method, all three doses of cocaine (0.125, 0.25, and 0.5 mg/injection) were tested in descending order during a single test session; each dose was available for 8 injections. A synopsis of the multi-dose method is given in Table I. The reproducibility of cocaine dose-response curves collected with the multi-dose method was assessed by testing five rats three times with this method. The effect of pretreatment with SCH 23390 or saline on the rate of cocaine self-administration was determined

using the multi-dose method in five rats. SCH 23390 (0.05 mg/kg) or saline was injected i.p. in a volume of 1 ml/kg 30 min before a multi-dose test session. For each of these three studies, rats were maintained on baseline training conditions (15 injections of 0.25 mg/0.1 ml) in between each multi-dose testing until they met the criterion of stability.

Experiment 3: Tolerance to self-administration of cocaine assessed by the multi-dose method. Six rats were placed in operant chambers equipped for chronic treatment for a period of 7 days. Levers were kept retracted throughout this period. The rats were randomly assigned to one of two groups: one group (n = 3) was infused with cocaine. Infusion sessions took place three times/day (08:00 h, 16:00 h, 12:00 h) with 20 doses of 0.25 mg of cocaine in 0.1 ml, with a 1-min inter-infusion interval. The twentieth infusion of cocaine was followed by a 0.2 ml saline-vehicle flush, and this flush was repeated every 2 h until the start of the next session. The other group (n = 3) was infused with saline-vehicle instead of cocaine, using exactly the same method. Twenty-four hours following the last series of infusions, subjects from both groups were tested using the multi-dose method as described previously. Rats were then left in their home cages for 5 days, after were returned to training on the 0.25 mg/inj baseline dose of cocaine. After rates of cocaine intake were within 20% of the pre-chronic

baseline for each subject, the experiment was repeated, except that chronic treatment conditions were crossed-over.

Data presentation and statistics. For all training and test sessions, data were scored as the average time occurring between reinforcers (the inter-reinforcer time; IS^RT, in min) without including the 30-s time-out that followed the delivery of each reinforcer. For training sessions and single-dose testing the time between the start of the session and self-administration of the first reinforcer was not included in the data analysis; the subsequent 14 IS^RT were used for data analysis. The time to the first reinforcer was not included in data analysis because this time was more variable than subsequent IS^RTs. For the multi-dose method, only the last 7 IS^RTs for each dose of cocaine were used for data analysis (see Table 1 for more detail).

Data from Experiment 1 were analyzed using a two-way ANOVA with repeated measures within the treatment condition. Data from Experiments 2 and 3 were analyzed using two way ANOVAs for repeated measures. In addition, following significant main effects of treatment (baseline, chronic cocaine of chronic saline-vehicle) for Experiment 3, pairs of treatments were compared by two-way ANOVA for repeated measures. All ANOVAs were performed with SYSTAT software (Wilkinson, 1990).

Drugs. Cocaine HCl (National Institute on Drug Abuse, Research Triangle Park, NC) was dissolved in heparinized saline (0.5 units/ml) and filtered through 0.22 μm filters (Millipore, Bedford, MA) into sterile 10-ml syringes immediately before use. SCH 23390 HCl (Research Biochemicals, Inc. Natick , MA) was prepared freshly before each test session and diluted in 0.9% sterile saline.

RESULTS

Experiment 1: Acquisition and loss of tolerance to cocaine. For both the Infused and Self-Administered groups, chronic cocaine treatment resulted in an orderly decrease in the IS^RT by day 2, with a maximum decrease to about 60% of baseline value appearing at day 4 and sustained until day 10 [$F_{(5,60)}$ = 1 1.53, $p < 0.0001$]; Fig. 1. When chronic cocaine was terminated, IS^RT values returned to base line levels within 6 days. IS^RT values recorded during and after termination of chronic cocaine treatment were not significantly different whether cocaine was infused of self-administered.

Experiment 2: Multi-dose method: Comparison with single-dose method, reproducibility, and effect of pretreatment with SCH 23390. There was no significant difference between the IS^RT values obtained the single or multi-dose

method (Fig. 2, top panel). For both methods, increasing the dose of cocaine form 0.125 to 0.25 to 0.5 mg/injection resulted in an orderly increase in the average IS^RT from approximately 2 to 3.5 to 6.5 min, respectively [$F_{(2,18)} = 213.03$, $P < 0.0001$]. Determination of cocaine dose-response curves during three consecutive multi-dose test sessions yielded almost superimposable curves, with no significant difference between the IS^RT values obtained during the first, second and third multi-dose test session (Fig. 2 middle panel). Pretreatment with SCH 23390, as compared to saline, decreased the IS^RT across all three doses of cocaine tested [$F_{(1,4)} = 19.18$, $P = 0.01$], resulting in nearly a twofold shift of the cocaine dose-response curve to the right (Fig. 2, bottom panel).

Experiment 3: Tolerance to self-administration of cocaine assessed by the multi-dose method. Chronic cocaine treatment decreased the IS^RT's across all three doses of cocaine tested [$F_{(2,10)} = 8.10$, $P< 0.01$; Fig. 3]. Post-hoc analysis showed that chronic cocaine was significantly different from both chronic saline [$F_{(1,5)} = 30.85$, $P< 0.01$] and from baseline [$F_{(1,5)} = 10.51$, $P< 0.02$]. IS^RTs obtained under baseline or chronic saline-vehicle conditions did not differ significantly. Chronic cocaine, as compared to chronic saline, shifted the cocaine dose-response curve nearly two-fold to the right.

TABLES

Table. 1. Multi-dose method. For clarity, letters and symbols have been replaced by dots for the last half of the medium dose and the entire low dose sections. For the medium and low dose sections, the entire sequences for 'Activated pump' , 'Solution infused' and 'IS^{R}T number' were constructed on the same principle as the one used for the high dose.

	High Dose								Medium Dose				Low Dose			
Activated Pump[a]	H	H	H	H	H	H	H	H	M	M	M	...	L	L	L	...
Solution Infused[b]	M/H	H	H	H	H	H	H	H	H/M	M	M	...	M/L	L	L	...
IS^{R}T number[c]	1	2	3	4	5	6	7	8	1	2	3	...	1	2	3	...

[a]Pump/syringe that was activated to deliver cocaine solution: H = dose (0.5 mg/injection); M = medium dose (0.25 mg/injection); L = low dose (0.125 mg/injection). At the beginning of the session, the catheter/line dead space (approx. 0.12 ml) was filled with medium dose. The priming injection (0.13 ml of the medium dose solution) was pushed into the catheter by delivery of the same volume of the high-dose solution. This resulted in the delivery of 0.13 ml of a mixed solution (M/H in 'Solution Infused' line below), containing 0.12 ml of medium dose and 0.01 ml of high dose and which left the catheter/line filled with the high-dose solution. Similarly, the last dose delivered for each set of dose of cocaine was driven by the next pump in the sequence for 0.13 ml volume. All other injections were delivered in a volume of 0.10 ml.

[b]Dose of cocaine delivered. M/H and H/M indicate that the solution of cocaine was a mixture of the high and medium doses; M/L indicate that the solution of cocaine was a mixture of the medium and low doses.

[c]Each dose of cocaine yielded 8 IS^RTs of which the last 7 were used for data analysis. The first IS^RT for each of the 3 doses was not used because it followed the delivery of a cocaine solution that contained a mixture of 2 doses of cocaine (i.e. M/H).

FIGURES

Figure. 1.

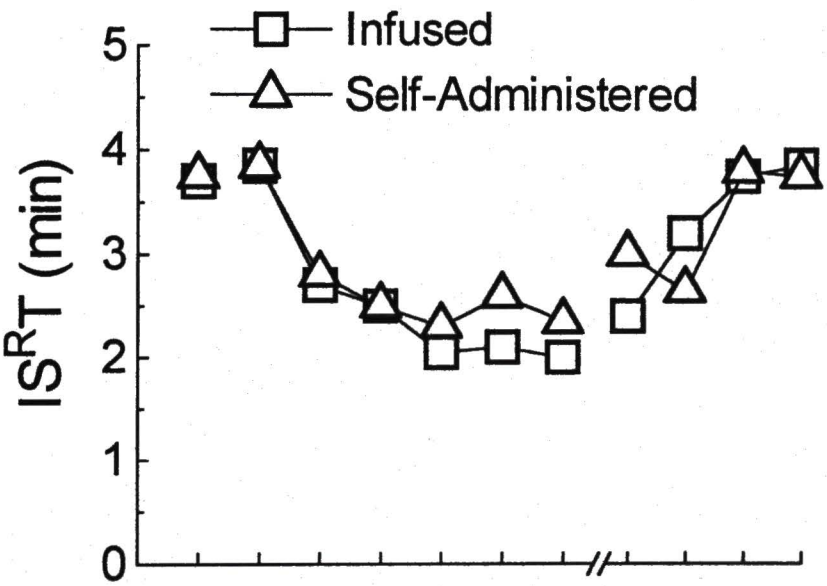

Fig. 1. Effect of infused of self-administered chronic cocaine regimen on the inter-reinforcer time. *Abscissa*: days during chronic cocaine regimen (20 mg/kg per 8 h) to the left of the break and days post-chronic cocaine regimen to the right of the break and days post-chronic cocaine regimen to the right of the break; base refers to baseline data that were obtained by averaging the IS^RTs obtained during the three self-administration sessions that immediately preceded the chronic cocaine regimen. Ordinate: inter-reinforcer time (IS^RT, in min). Open squares indicated infusion of chronic cocaine (20 injections of 0.25 mg cocaine, spaced one min apart; n = 9); open triangles indicate self-administration of chronic cocaine (20 self-administered injections of 0.25 mg cocaine; n = 5). Symbols represents group means. For clarity, S.E.M. bars were omitted. S.E.M.s averaged 0.23 and 0.30 for the Infused and Self-Administered groups, respectively.

Figure. 2.

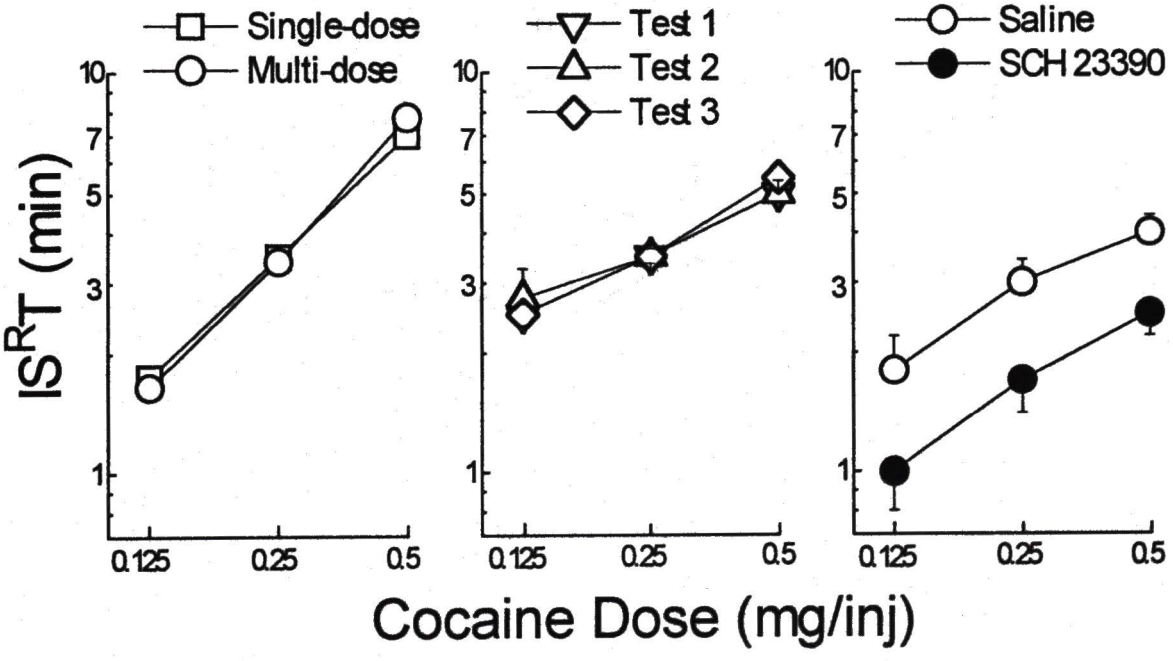

Fig 2. Multi-dose method: comparison with single-test method, reproducibility and effect of pretreatment with SCH 23390. Abscissa: dose of cocaine (mg/injection). Ordinate: inter-reinforcer time (IS^RT, in min) in logarithmic scale. Top panel: comparison of cocaine dose-response curves using single-dose (open squares) or multi-dose (open circles) method (n = 10). Middle panel: Reproducibility of cocaine dose-response curves using the multi-dose method. Down triangles indicate the first, up triangles indicate the second and diamonds indicate the third multi-dose test (n=5). Bottom panel: Effect of SCH 23390 or saline pretreatment on the cocaine dose-response curve using the multi-dose method. Saline (open circles) or SCH 23390 (0.5 mg/kg; filled circles) as injected IP 30 min pre-session (n = 5). In all panels data are presented as group means ± S.E.M. (some S.E.M. bars are smaller than the symbols).

Figure. 3.

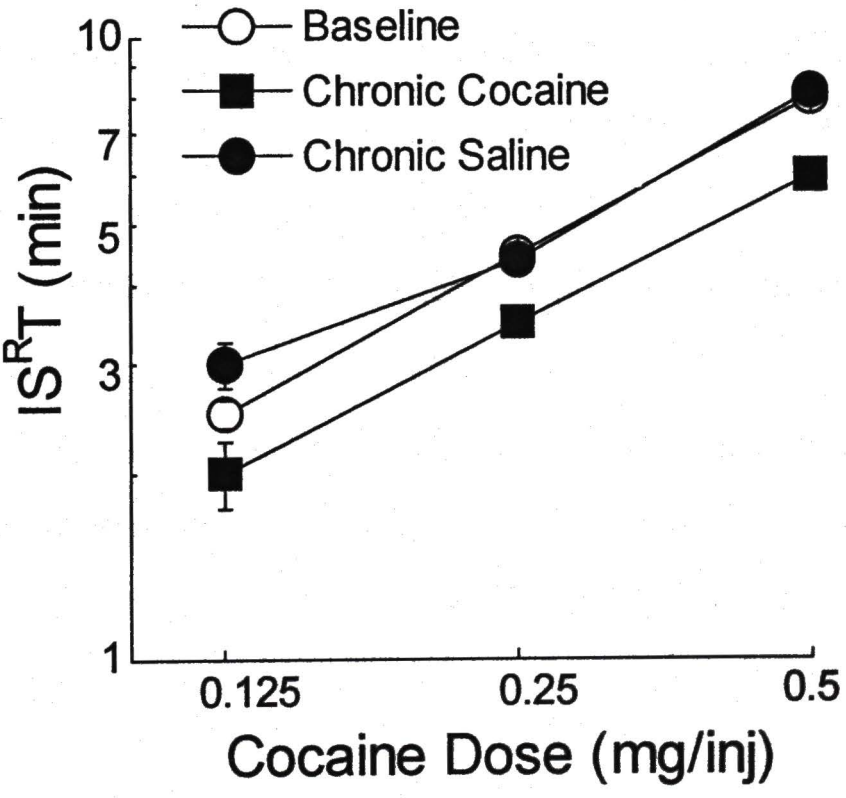

Fig. 3. Tolerance to self-administration of cocaine using the multi-dose method. Refer to Fig 2 for description of abscissa and ordinate. All three dose-response curves were obtained using the multi-dose method. Circles indicate the dose-response curve for cocaine in the absence of chronic treatment (baseline); filled triangles indicate the dose-response curve obtained 24-h after chronic treatment with cocaine; filled squares indicate the dose-response curve obtained 24-h after chronic treatment with saline-vehicle. Chronic cocaine or saline treatments were obtained in the same rats using a crossover design (n = 6). Chronic cocaine (20 mg/kg per 8 h) or saline-vehicle (20 injections of 0.1 ml each every 8-h) treatment was administered for 7 days. Data are presented as means ± S.E.M.

DISCUSSION

In the present study, during a 10-day regimen of cocaine, 20 mg/kg per 8 h, the onset of tolerance was very rapid; within 48 h, tolerance was nearly maximum. Once acquired, tolerance remained stable from day 6 through day 10 of chronic administration. When chronic cocaine was terminated, the offset of tolerance was slower than the onset, requiring approximately 4-6 days. In our previous report of tolerance to the effects of cocaine (Emmett-Oglesby and Lane, 1992), those subjects received 20 mg/kg per 8 h of cocaine for a week, and tolerance was found to extend over 4 days following termination of chronic cocaine. The time course of tolerance loss in the present experiment is consistent with this previous result.

Three mechanisms might account for the development of tolerance to cocaine in a self-administration paradigm: tolerance could occur via pharmacokinetic mechanisms; tolerance could occur to the behavior-disrupting effects of cocaine; or tolerance could occur to the reinforcing efficacy of cocaine. Pharmacokinetic factors, such as increased rate of elimination of cocaine, could decrease the plasma concentration of cocaine, resulting in faster intake; however, this hypothesis is unlikely to be correct because chronic cocaine administration has been reported not to change cocaine's half-life of elimination (Misra, 1976). We are nonetheless currently undertaking a series of experiments to assess this hypothesis. The second possibility, tolerance to the behavioral-disrupting effects of cocaine, stems from the hypothesis that rate of drug self-administration is limited by behavior-disrupting effects of the reinforcer. According to this hypothesis, subjects would take more drug per unit time simply because they are physically capable of taking more drug. This second hypothesis leads to an interesting prediction concerning the lowest dose of cocaine that subjects self-administer. If indeed the shorter IS^RTs observed following chronic treatment with cocaine were due to tolerance to the behavior-disrupting effects, then one would expect to see that a low dose of cocaine, which barely maintains cocaine self-administration under baseline conditions, would result in greater cocaine intake following chronic treatment with cocaine. Instead, the entire dose-effect curve was shifted nearly twofold to the right by chronic cocaine, and the lowest dose that barely maintained self-administration before chronic cocaine was not self-

administered after chronic cocaine (Emmett-Oglesby and Lane, 1992). In addition, the role of behavior-disruptive effects in the control of drug self-administration is minimized by the observation that are capable of emitting operant responses for alternative reinforcers during the time between self-administration injections of psychostimulants (Wise *et al.*, 1977). The third hypothesis, that tolerance occurs to the reinforcing effect of cocaine, is consistent with both our previous results (Emmett-Oglesby and Lane, 1992) as well as data from the present study.

In humans, tolerance to cocaine has been observed to occur within a single session of use (Fischman *et al.*, 1985; Ambre *et al.*, 1988), but no tolerance has been seen from one session of use to another when 24-h separated sessions (Fischman *et al.*, 1985). In the present experiments, tolerance persisted for at least 4 days after termination of chronic cocaine. As compared to human experiments, where cocaine dosing typically lasts for only a single session, the present experiment used a 10 day treatment regiment with drug administered every 8 h. The development of tolerance is known to be dependent upon critical parameters such as the dose of drug administered, the dosing interval, and the duration of dosing (Kalant *et al.*, 1971). Thus, the human studies may have failed to show tolerance perhaps because cocaine was not given at high enough a dose, not frequently enough and / or for too short a duration.

Substantial differences in metabolism of brain neurotransmitters are associated with self-administered versus non-contingently infused opioids (e.g., Smith and Lane, 1983). Recently, changes in serotonin metabolism in rat brain have been reported to occur depending on response-contingency of cocaine administration (Dworkin, 1992). If these neurochemical differences are critical for the development of tolerance, then in the present experiment, we should have seen a difference in the rate of development or magnitude of tolerance when chronic cocaine was self-administered versus experimenter-infused. Since the mode of administration of cocaine had no differential effect, response contingency is not likely to be a critical variable for the development of tolerance to the reinforcing effects of cocaine, at least for the dose of cocaine that we investigated (0.25 mg/injection).

Two factors might confound this conclusion. First, the determine the rate and extent of tolerance development, the infused group were in fact self-administered cocaine every second day, and one could argue that both groups were in fact self-administering the drug,. although to a different extent. This explanation is, however, unlikely to be correct: tolerance had already developed in the Infused group when tested for the first time at day 2, so the occurrence of tolerance was not dependent upon self-administration of cocaine in this group. Further, a similar degree of tolerance has been obtained in subjects who received 7 days of cocaine, 20 mg/kg per 8 h, by infusion only, before being tested

for tolerance (Emmett-Oglesby and Lane, 1992). Thus, in the present study, the fact that subjects in the infused group were allowed to self-administer once every 2 days does not alter the conclusion that contingent versus non-contingent cocaine administration is not a significant issue in the development to tolerance. A second factor that might confound the issue of contingency of drug administration is the difference in the rate at which chronic cocaine was infused (once per minute for 20 injections) vs. self-administration (from day 2 onwards, approximately once every 2.3 min). It is possible that a different result might have been obtained had injections for the two groups been yoked. However, 2.3 min versus 1 min inter-injection intervals results in a 46 vs. 20-min total time of drug injection for the two groups, which Is 9% vs. 4% of an 8-h period. This relatively small difference is unlikely to be important since comparable degrees of tolerance were observed between the two groups.

A second aim of these experiments was to develop a method that would permit more rapid collection of dose-response data in a single session. In the present experiment, we modified their method for use in rats and, therefore, we first compared data obtained from the multi-dose method with data obtained from our single-dose method. The two methods produced essentially interchangeable dose-response curves, and to our knowledge this is the first demonstration that single-dose and multi-dose testing methods produce comparable data. The concordance of data obtained between the multi-dose and single-

dose methods was further demonstrated by the shift to the right of the cocaine dose response curve that occurred following 0.05 mg/kg SCH 23390. SCH 23390 is well characterized as being able to block the reinforcing effects of cocaine in both primates (Woolverton, 1986; Bergman *et al.*, 1990) and rats (Koob *et al.*, 1987; Hubner and Moreton, 1991; Lane *et al.*, 1992). In the present experiment, SCH 23390 produced nearly a two-fold shift to the right of the cocaine dose-response curve, which was essentially identical to the shift observed when SCH 23390 was tested in this laboratory using our single-dose method (Lane *et al.*, 1992). Finally, the multi-dose method appears to be reliable with regard to replication of data across tests, since three repetitions of the cocaine dose-response curve produced essentially interchangeable results. Thus, this method yields reproducible data, and it does so in a much shorter time than is required by single-dose methods.

The multi-dose method in these experiments was accomplished by using different syringes for each dose of cocaine, and switching from one syringe to another to change doses delivered. This method produces a constant volume and flow rate for all injections, which can be contrasted to the method of Winger *et al.* (1989), who delivered different doses of cocaine by using a single syringe and varying the volume of drug solution that was injected. In the present study, we were concerned that such a procedure might produce very low (or very high) injection volumes, which could modify cocaine absorption. Consequently, we

elected to use multiple pumps and maintain all injections at a fixed volume of 0.1 ml.

Typically, tolerance is assessed after some period of chronic treatment. If dose-response determinations are to be performed using a single-dose method, the experimenter is often faced with an unpleasant trade-off. On the one hand, if the power of a within-subjects design is to be exploited, then dose-response testing must occur over days; thus, if tolerance is waxing or waning over days, dose-response data may be influenced by the longitudinal nature of these studies. On the other hand, a between-subjects design, which permits testing of all doses in a single day, requires a substantial increase in the number of subjects to be tested. In the present experiment, the multi-dose method enabled us to use a within-subjects design to obtain, during a single session, a cocaine dose-response curve following chronic treatment with cocaine or saline-vehicle. The effect of chronic cocaine, 20 mg/kg per 8 h for 7 days produced no significant change from the baseline dose-response data. These data extend findings reported by Emmett-Oglesby and Lane (1992), who used single-dose testing methodology and found approximately the same magnitude of tolerance to self-administered cocaine following this same dosing regimen of chronic cocaine. Thus, the multi-dose method appears to be particularly useful in studies of tolerance.

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CHAPTER 2

The experiments in this chapter were designed to test the following-specific aims: 3) To determine if IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) will produce tolerance comparable to IV administration of cocaine; and 4) to determine if the IP administration of chronic cocaine (20 mg/kg/8 hr for 7 days) produces pharmacokinetic tolerance to the reinforcing effects of cocaine as demonstrated by reduced plasma and/or brain concentrations of cocaine and benzoylecgonine (BZE). The hypotheses being tested are that the chronic IP administration of cocaine (20 mg/kg/8 hr for 7 days) will result in a shift of the dose-response curve for cocaine self-administration to the right, indicating tolerance to the reinforcing effects of cocaine; and that chronic IP administration of cocaine (20 mg/kg/8 hr for 7 days) will produce a decrease in the concentration of cocaine in the plasma and / or brain indicating pharmacokinetic tolerance to cocaine. A final form of this manuscript will be submitted to *Pharmacol. Biochem. Behav.*

Tolerance to the reinforcing effects of cocaine is not due to pharmacokinetic factors

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Abbreviations:

Cocaine (COC)

injection (inj)

inter-reinforcer time (IS^RT)

ABSTRACT

These experiments tested the hypothesis that the chronic administration of cocaine (COC) produces tolerance to the reinforcing effects of cocaine by a pharmacokinetic mechanism. Rats (N=57) were implanted with indwelling jugular catheters. In the first experiment, rats (n=10) were trained to self-administer cocaine under a fixed-ratio 2 (FR2) schedule of reinforcement. Dose-response curves were obtained before rats were treated chronically with COC (20 mg/kg/8 hr/7 days; IP) or saline and twenty-four hours after termination of COC treatment. Chronic COC administration resulted a significant shift of the dose response curve indicating tolerance to the reinforcing effects of cocaine. In the second experiment (n=19), a pre-chronic time-course of cocaine clearance was determined. The rats were then treated chronically with either COC (20 mg/kg/8 hr/7 days; IP) or saline, and a post-chronic time-course of cocaine clearance was determined. Gas Chromatography / Mass Spectroscopy (GCMS) analysis showed no significant differences in the pre- and post-chronic blood concentrations of benzoylecgonine (BZE), a major metabolite of COC, indicating that the chronic treatment with COC did not alter the biotransformation of COC. In the third experiment (n=28), the levels of pre-chronic COC and BZE in whole blood samples were measured 15 min before and after an IV injection of COC (2.0 mg/kg). For one group of rats (n=9), the brains were removed immediately after the second blood sample was taken. The other rats (n=17) were then treated

chronically with either COC (20 mg/kg/8 hr/7 days; IP) or saline. Twenty-four hours following the final chronic injection, whole blood samples were collected 15 min prior to and 15 min after an IV injection of COC (2.0 mg/kg). The brains of these subjects were removed immediately after the second blood sample was collected. Chronic COC treatment had no significant effect on the concentration of COC or BZE measured in blood or brain tissue after an IV injection of COC. In summary, chronic IP treatment with COC (20 mg/kg/8 hr/7 days) produces tolerance to the reinforcing effects of cocaine, which is not due to an increase in cocaine biotransformation or a reduction of COC distribution into the brain.

INTRODUCTION

Rats trained to self-administer cocaine under an FR2 schedule of reinforcement demonstrate an orderly increase in the time between injections as the dose of cocaine is increased. In other words, as the dose of cocaine increases, the rate of self-administration decreases. Cocaine given chronically produces tolerance by shifting the dose-response curve for cocaine self-administration to the right (Emmett-Oglesby and Lane, 1992; Depoortere *et al.*, 1993). What is not known is whether this chronic cocaine regimen is producing pharmacody-

namic or pharmacokinetic tolerance. Either scenario could explain why a chronic cocaine regimen results in a higher rate of cocaine self-administration. For example, if chronic cocaine is producing pharmacodynamic tolerance it could reduce the reinforcing properties of cocaine by decreasing the number of active dopamine receptors in the brain. If this is the case, then a given dose of cocaine would be perceived as a lower dose after the chronic regimen. Chronic cocaine could also be producing pharmacokinetic tolerance to cocaine by changing the rate at which cocaine is being transformed and excreted, or by modifying distribution of cocaine into brain. If this is the case, a given dose of cocaine would be self-administered at a faster rate because there would actually be less cocaine available at it's site of action.

The previous experiment demonstrated tolerance to the reinforcing effects of cocaine after IV administration of chronic cocaine (Emmett-Oglesby *et al.*, 1993). In that study, rats lived in the operant chambers during the chronic treatment regimen, and the cocaine was delivered IV through the jugular catheters at an infusion rate of 1.0 mg/kg/min for 20 min, which occurred every 8 hr for 7 days.

It has been suggested that the silastic polymer with which catheters are constructed may sequester cocaine if they are in contact with cocaine-containing solutions for extended periods of time (as is the case when chronic cocaine is delivered by the IV route). To assure that the jugular catheters have a limited

exposure to cocaine during the pharmacokinetic experiments, IP administration of chronic cocaine will be used. Therefore, the first experiment will be conducted to determine if IP administration of chronic cocaine will produce tolerance to the reinforcing effects of cocaine comparable to that produced by the IV route. In the following two pharmacokinetic experiments, the effect of this regimen of chronic cocaine (20 mg/kg/8h/7 days; IP) treatment will be determined by measuring blood and brain concentrations of COC and BZE (a metabolite of cocaine) using gas chromatographic / mass spectrometry (GCMS) at behaviorally relevant time points after IV injection of COC.

METHODS

Self-Administration.

Subjects. Male Fisher F-344 rats were housed singly and maintained at 270 g $\pm$ 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12:12 light:dark cycle.

Training. For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were

implanted with an indwelling catheter inserted into the right external jugular, the free end of which was fixed to the skull. After recovery, subjects were given the opportunity to self-administer cocaine on an FR1 schedule with a maximum of 25 cocaine infusions. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were switched to an FR2 training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing the catheter with 0.1 ml of heparinized saline.

The FR2 training schedule had a maximum of 15 reinforcers and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions.

Testing. Rats (n=10) were tested using a multi-dose procedure. With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/infusion) were available for self-administration during a single test session. This test session consisted of a standard priming infusion (0.3 mg) followed by 24 infusions. These 24 infusions were divided into three blocks of 8 infusions each, with the first block of eight reinforcers containing 0.5 mg/infusion of cocaine, the second containing 0.25 mg/infusion and the third containing 0.125 mg/infusion. Cocaine self-administration tests were conducted in a descending order of cocaine doses

(0.5, 0.25, 0.125 mg/infusion); testing was initiated with the high dose in order to increase the probability that the rats begin self-administration. Baseline dose-response curves for cocaine self-administration were obtained 24-hr before the chronic treatment. Twenty-four hours after the last chronic injection, post-chronic dose-response curves for cocaine self-administration were obtained.

Chronic Treatment Regimen. After baseline dose-response data were obtained, all training and testing were suspended. During this time, all rats received IP injections of COC (20 mg/kg/8 hr/7 days). Twenty-four hours after the last chronic injection of COC, post-chronic dose-response curves for cocaine self-administration were obtained.

Drugs. For self-administration, cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 µm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. For the chronic cocaine treatment, cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in saline and injected IP.

Data Analysis. In FR2 self-administration experiments, data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, $IS^{R}T$) and analyzed using a two-way repeated

measures ANOVA with treatment condition and dose of cocaine as within subject factors.

Blood data were analyzed using a 2 (treatment condition) x 2 (pre- or post-chronic) repeated measures ANOVA, with sample time as the repeated measure. Data from each brain section were analyzed using a 1-way between groups ANOVA, with blood concentration as a covariant. All data were analyzed using the SYSTAT statistical software package (Wilkinson *et al.*, 1992).

Pharmacokinetic Experiments.

Subjects. Male Fisher F-344 rats were housed singly and maintained at 270 g $\pm$ 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Sample Acquisition. All rats were implanted with jugular catheters as described above. After a five day recovery period, the sample acquisition and chronic COC treatment began.

Blood: Whole blood samples (200ul) were taken from each rat through the jugular catheter. After a sample was taken, the catheter was immediately flushed with an equal amount of saline. The blood samples were put into 2 ml of

distilled deionized H_20, vortexed and immediately frozen until they were analyzed.

Brains: The rats were decapitated and their brains removed. The brains were dissected into three gross sections: A- includes frontal cortex, nucleus accumbens, and striatum; B- hypothalamus, thalamus, motor cortex, and hippocampus; and C- midbrain (including dopamine cell bodies) and brain stem. After dissection, the brains were frozen on dry ice and stored in a -70° freezer until they were analyzed.

Blood and Brain Analysis.

Blood: Whole blood samples were thawed and internal standard, consisting of a phosphate buffer and a fixed amount of d3-cocaine and d3-benzoylecgonine, were added. The resulting mixture was centrifuged for 10 minutes at 2000 rpm. The supernatant was passed through a "Clean Screen" filter to absorb COC and BZE and the filter was washed with 2 ml each of distilled water, dilute HCl and methanol. The COC and BZE was then eluted from the filter with 3 ml of methyl chloride/isopropyl alcohol/NH_4OH (78/20/2 % respectively) and evaporated to dryness at $\leq$ 40° C. The residue was dissolved by adding 50µl of ethyl acetate and 50 µl BSTFA was added to form detectable derivatives. The resulting solution was sealed in glass sample tubes and heated

for 20 minutes at 70° C, then allowed to cool. Once the samples reached room temperature, aliquots of each sample were analyzed using a Gas Chromatograph / Mass Spectrometer (GCMS). Data are expressed as ng / ml blood.

Brains: The brains sections were thawed, 2 ml of distilled deionized H_20 was added and they were homogenized by sonication (Branson) at 4°C. Internal standard was added and the resulting mixture was centrifuged for 10 minutes at 2000 rpm. The brain samples were processed and analyzed using the exact same procedure described above for the blood samples. Data are expressed as ng / g tissue.

RESULTS

In the self-administration experiment, increasing the dose of cocaine resulted in an orderly increase in the time between injections ($F_{(2,18)} = 43.35$; $p < 0.001$) (Figure 1). Chronic treatment with COC (20 mg/kg/8 hr for 7 days, IP; Figure 1) shifted the cocaine self-administration curve significantly to the right ($F_{(1,9)} = 11.176$; $p < 0.01$).

In the initial pharmacokinetic experiment, chronic COC treatment failed to increase the blood concentration of BZE at any time point tested (Fig. 2, bottom

panel). There was an overall effect of chronic COC treatment on the concentration of COC in the blood; however, there was an interaction with time such that COC was higher in chronic COC treated animals only at 15 min after the IV injection of 2.0 mg/kg of COC ($F_{(1,134)} = 49.817$; $p < 0.001$; Fig. 2, top panel). This time point was replicated in the second pharmacokinetic experiment. This replication demonstrated that this effect was not reproducible and an analysis of the pooled data from this time point showed no significant effects of chronic COC treatment (Fig. 3). In addition, chronic COC treatment, as compared to chronic saline, did not produce a significant change in the concentration of COC or BZE measured in brain sections (Fig. 4).

FIGURES

Figure 1

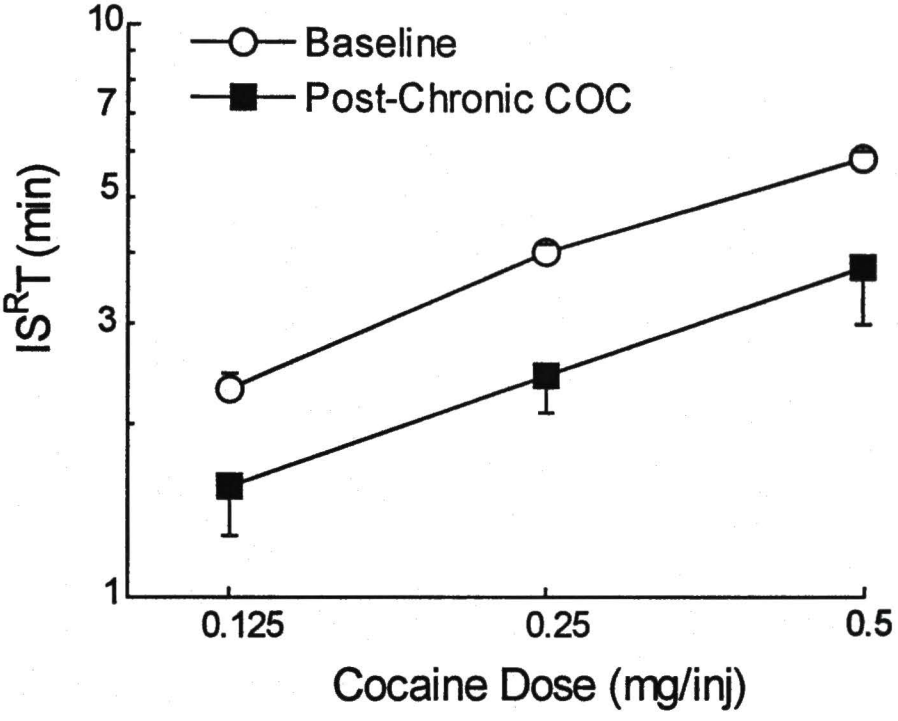

Fig.1: The effects of chronic treatment with COC (20 mg/kg/8 hr for 7 days; IP) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed circles indicate the dose-response curve for cocaine self-administration 24 hours after the last chronic injection of COC. Data are represented as group means (n=10) $\pm$ S.E.M.

Figure 2

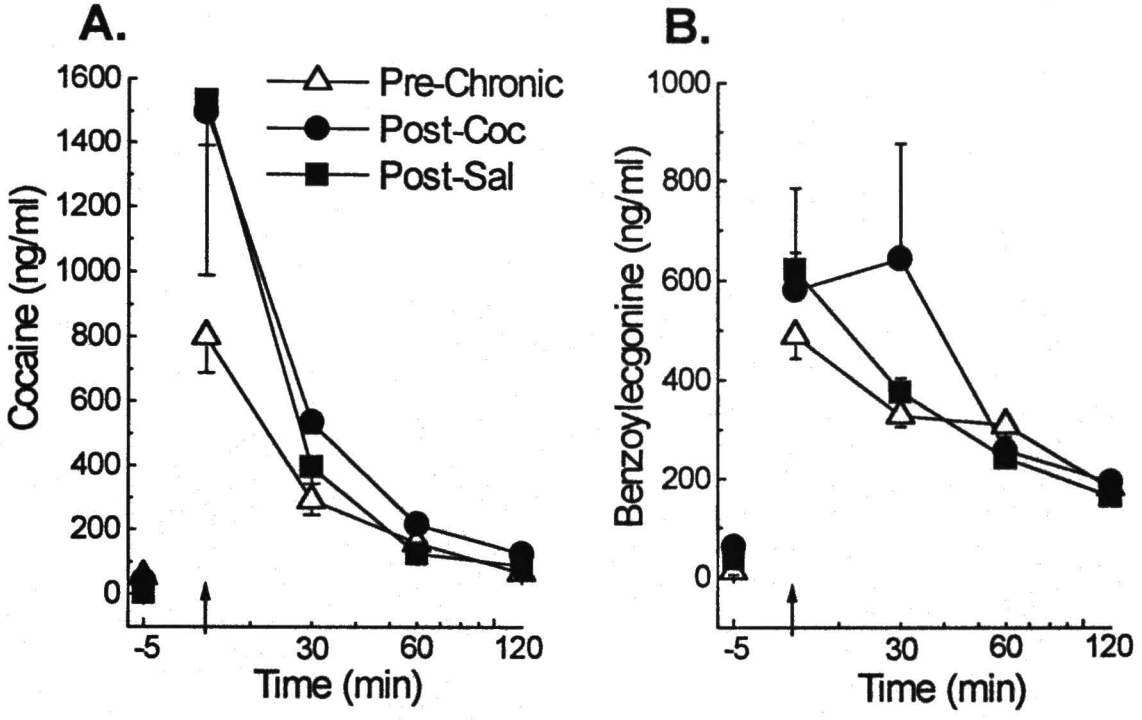

Fig. 2: The effects of chronic treatment with COC (20 mg/kg/8 hr for 7 days; IP) on cocaine clearance in whole blood. Abscissa: Time (minutes). Ordinate: Panel *A* = cocaine concentration (ng/ml); Panel *B* = benzoylecgonine concentration (ng/ml). Open triangles indicate the clearance curve for cocaine before chronic treatment (baseline). Closed circles indicate the clearance curve for cocaine self-administration 24 hours after the last chronic injection of COC. Closed squares indicate the clearance curve for cocaine self-administration 24 hours after the last chronic injection of saline. Arrows indicate an IV infusion of COC, 2.0 mg/kg. Data are represented as group means $\pm$ S.E.M.

Figure 3

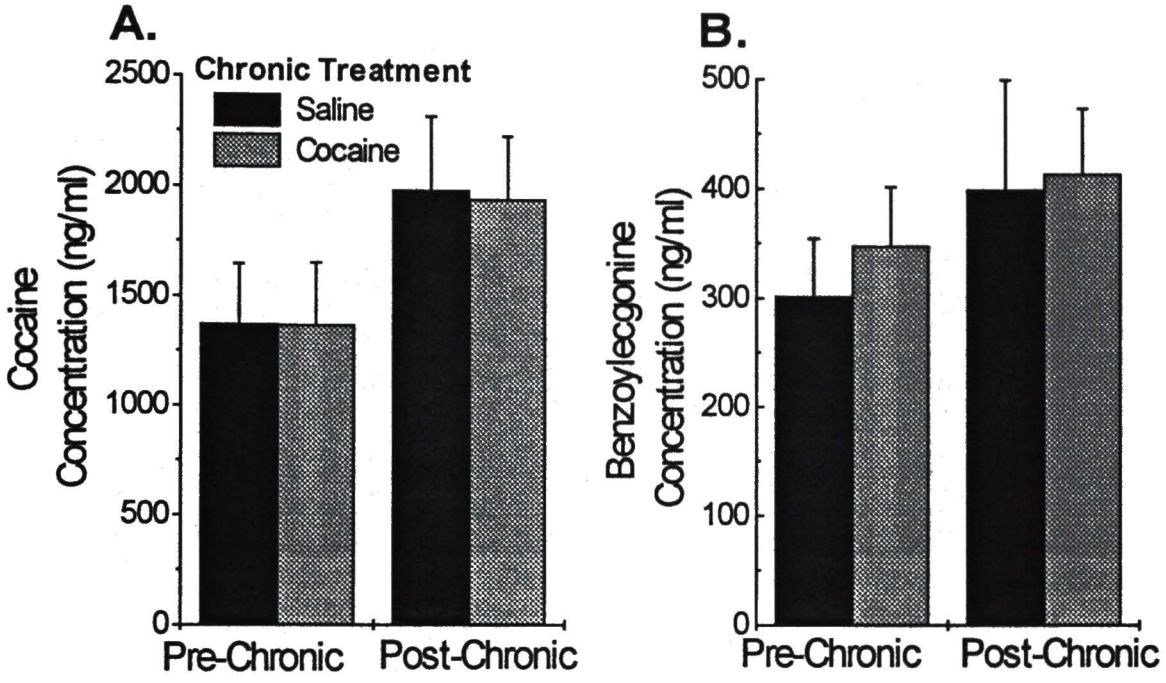

Fig. 3: The effects of chronic treatment with COC (20 mg/kg/8 hr for 7 days; IP) on cocaine (Panel *A*) and benzoylecgonine (Panel *B*) concentrations in the blood 15 min after an IV injection of COC. Abscissa: Pre- or post-chronic. Ordinate: concentration of cocaine or benzoylecgonine (ng/ml). Data are represented as group means ± S.E.M.

Figure 4

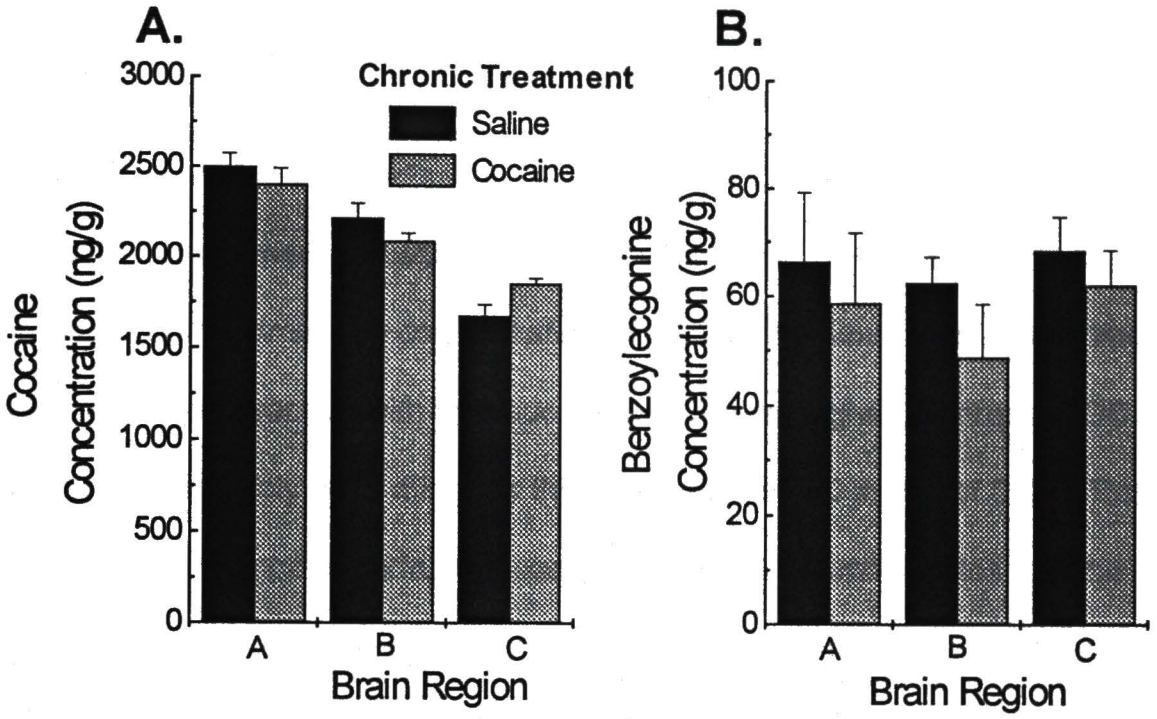

Fig. 4: The effects of chronic treatment with COC (20 mg/kg/8 hr for 7 days; IP) on cocaine (Panel *A*) and benzoylecgonine (Panel *B*) concentrations in brain. Abscissa: Brain sections. Ordinate: concentration of cocaine or benzoylecgonine (ng/g). Data are represented as group means ± S.E.M.

DISCUSSION

Chronic IP treatment with COC (20 mg/kg/8 hours for 7 days) produced a two-fold shift to the right of the dose-response curve for cocaine self-administration, indicating tolerance to the reinforcing effects of cocaine. These

results are comparable to those obtained previously in this laboratory employing IV administration of chronic COC (Emmett-Oglesby *et al.*, 1993).

Chronic COC treatment failed to increase the blood concentration of BZE at any time point tested, indicating that chronic COC did not produce an increase in the biotransformation of cocaine. Though there was an overall effect of chronic COC treatment on concentration of COC in the blood in this experiment there was an interaction with time such that COC was higher in chronic COC treated animals only at 15 min after the IV injection of 2.0 mg/kg of COC. Replication of this time point during the second experiment demonstrates that this was not a reproducible effect and the pooled data for this time point indicate no effect of chronic COC treatment on the concentration of COC in blood. In addition, chronic treatment with COC did not change either the volume of distribution of cocaine (3 l/kg) or the t1/2 for clearance (24 min). These values are consistent those reported by Stewart *et al.*, 1978.

Chronic COC treatment, as compared to chronic saline, did not produce a significant change in the concentration of COC or BZE measured in brain sections.

Previous studies have reported either no differences or small differences in plasma (Katz *et al.*, 1993; Nayak *et al.*, 1976) and brain (Ferko *et al.*, 1990) levels of cocaine after repeated administration of cocaine. There have also

been reports that after IP administration, concentrations of COC in the brain of chronic-COC treated rats were significantly higher than after an acute dose (Ho *et al.,* 1977; Petit et al., 1990). These reports and our current data do not support the hypothesis that the tolerance to the reinforcing effects of cocaine is due to pharmacokinetic factors since there is no reduction of cocaine available at the site of action.

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CHAPTER 3

The experiments in this chapter were designed to test the following specific aims: 5) To determine if the *acute* administration of either direct or indirect DA *agonists* will augment the reinforcing effects of cocaine; 6) To determine if the *acute* administration of direct DA *antagonists* block the reinforcing effects of cocaine. The hypotheses are 1) the acute administration of dopamine agonists will mimic the effects of cocaine and decrease the rate of cocaine self-administration; 2) the acute administration of dopamine antagonists will block the reinforcing effects of cocaine and increase the rate of cocaine self-administration. These experiments were conducted to demonstrate that the action of dopamine agonists and antagonists on cocaine's reinforcing effects could be detected in the multi-dose paradigm of cocaine self-administration using the IS^RT as the dependent variable.

Acute dopamine agonists and antagonists: Effects on cocaine self-administration in rats

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Running Title: Dopaminergic Compounds and Cocaine self-administration.

Abbreviations:

d-amphetamine (*d*-A)

methamphetamine (METH)

apomorphine (APO)

flupenthixol (FLU)

Eticlopride (ETI)

SCH23390 (SCH)

fixed ratio (FR)

injection (inj)

inter-reinforcer time (IS^RT)

ABSTRACT

These experiments tested the hypothesis that the acute administration of the dopamine agonists apomorphine (APO), *d*-amphetamine (*d*-A), and methamphetamine (METH) will augment the reinforcing effects of cocaine, while the acute administration of dopamine antagonists flupenthixol (FLU), SCH23390 (SCH) and eticlopride (ETI) will attenuate the reinforcing effects of cocaine. Rats (N=36) were implanted with indwelling jugular catheters and were trained to self-administer cocaine under a fixed-ratio 2 (FR2) schedule of reinforcement. After stable patterns of self-administration were observed, baseline dose-response curves were obtained. Test compounds were then administered subcutaneously 30 min prior to a dose-response determination for cocaine self-administration. Only one dose of the indirect dopamine agonist METH (1.8 mg/kg) produced a significant decrease in the rate of cocaine self-administration. In contrast, the indirect dopamine agonists *d*-A (0.32, 1.0, and 3.2 mg/kg) and METH (0.32, 1.0 and 3.2 mg/kg), as well as the direct dopamine agonist APO (0.32, 1.0 and 3.2 mg/kg) failed to significantly alter the dose-response curve for cocaine self-administration. However, it is worth noting that at high doses of these agonists produced a large delay in onset of cocaine self-administration. In contrast, the nonspecific dopamine antagonist FLU (0.032, 0.1 and 0.32 mg/kg) as well as the specific D1 antagonist SCH (0.0032, 0.01 and 0.032 mg/kg) and the specific D2 antagonist ETI (0.0032, 0.01 and 0.032 mg/kg) all produced a

dose-dependent increase in the rate of cocaine self-administration. These data provide evidence for the involvement of both the D1 and D2 receptor in the reinforcing properties of cocaine.

INTRODUCTION

The reinforcing effects of cocaine have been extensively studied and appear to be mediated, at least in part by the mesocorticolimbic dopamine system (Goeders and Smith, 1983, 1986; Post *et al.*, 1987; Roberts and Koob, 1982; Roberts *et al.*, 1977, 1980). A close correlation exists between the ability of cocaine-like compounds to bind to the dopamine uptake site and their potency in self-administration paradigms (Ritz *et al.*, 1987). A unique property of drug self-administration, is that animals maintain a relatively stable level of drug intake over time (Pickens *et al.*, 1978; Yokel and Pickens, 1974). For example, animals respond to changes in the injection dose by increasing their rate of self-administration following decreases in dose and decreasing their rate of self-administration following increases in the injection dose (Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Goldberg *et al.*, 1971; Pickens and Thompson, 1968; Wilson *et al*, 1971; Woods and Schuster, 1968).

Dopamine antagonists are thought to block the reinforcing effects of cocaine, because increased rates of cocaine self-administration are produced by pretreatment these compounds (Britton *et al.,* 1991; Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Ettinberg *et al.*, 1982; Roberts and Vickers, 1984). In contrast, acute pretreatment with the indirect dopamine agonist *d*-amphetamine results in a decrease in the rate of either cocaine or *d*-amphetamine self-administration (Pickens *et al.*, 1968; Wilson and Schuster, 1973).

As I mentioned above, there are few reports in the literature describing the effects of dopamine agonists on cocaine self-administration. These reports; however, have only examined the effects of *indirect* dopamine agonists. Therefore, the present experiment was designed to replicate and extend these results by examining the effects of *direct* (apomorphine, APO) and *indirect* (*d*-amphetamine, *d*-Amp; and methamphetamine, METH) dopamine agonists on cocaine self-administration. In addition, these experiments will also compare the effects of the mixed dopamine antagonist flupenthixol (FLU) with the specific D1 antagonist SCH23390 (SCH) and the specific D2 antagonist eticlopride (ETI).

METHODS

Subjects. Subjects, male Fisher F-344 rats (n=36), were housed singly and maintained at 270 g ± 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training. For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were implanted with indwelling catheters inserted into the right external jugular, the free end of which was fixed to the skull. After recovery, subjects were given the opportunity to self-administer cocaine on an FR1 schedule with a maximum of 25 cocaine injections. Once rats self-administered all 25 injections within 3 hr, during two consecutive training sessions; they were then switched to an FR2 training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

The FR2 training schedule had a maximum of 15 reinforcers and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-

reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions.

Testing. After the stability criterion was met, baseline dose-response curves for cocaine self-administration were obtained using a multi-dose procedure. With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/injection) were available for self-administration during a single test session. This test session consisted of a standard priming injection (0.3 mg) followed by 24 injections. These 24 injections were divided into three blocks of 8 injections each, with the first block of eight reinforcers containing 0.5 mg/injection of cocaine, the second containing 0.25 mg/injection and the third containing 0.125 mg/injection. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/injection); testing was initiated with the high dose in order to increase the probability that the rats begin self-administration.

Acute Testing. After baseline dose-response data were obtained, the acute testing phase began. During this time, all rats were tested with either APO (0.32, 1.0 and 3.2 mg/kg), *d*-A (0.32, 1.0, 1.8 and 3.2 mg/kg), METH (0.32, 1.0 and 3.2 mg/kg), FLU (0.032, 0.1 and 0.32 mg/kg), SCH (0.0032, 0.01 and 0.032 mg/kg) or ETI (0.0032, 0.01 and 0.032 mg/kg). Each subject was tested with only one of the dopaminergic compounds; however, subjects were tested with all doses of that test compound in a random order. For the test sessions, subjects

reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions.

Testing. After the stability criterion was met, baseline dose-response curves for cocaine self-administration were obtained using a multi-dose procedure. With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/injection) were available for self-administration during a single test session. This test session consisted of a standard priming injection (0.3 mg) followed by 24 injections. These 24 injections were divided into three blocks of 8 injections each, with the first block of eight reinforcers containing 0.5 mg/injection of cocaine, the second containing 0.25 mg/injection and the third containing 0.125 mg/injection. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/injection); testing was initiated with the high dose in order to increase the probability that the rats begin self-administration.

Acute Testing. After baseline dose-response data were obtained, the acute testing phase began. During this time, all rats were tested with either APO (0.32, 1.0 and 3.2 mg/kg), *d*-A (0.32, 1.0, 1.8 and 3.2 mg/kg), METH (0.32, 1.0 and 3.2 mg/kg), FLU (0.032, 0.1 and 0.32 mg/kg), SCH (0.0032, 0.01 and 0.032 mg/kg) or ETI (0.0032, 0.01 and 0.032 mg/kg). Each subject was tested with only one of the dopaminergic compounds; however, subjects were tested with all doses of that test compound in a random order. For the test sessions, subjects

received a SC injection of the test compound 30 minutes prior to a cocaine self-administration test.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 µm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. APO, FLU, SCH, and ETI (Sigma, St. Louis, MO) were dissolved in water and injected SC. *d*-A and METH (Sigma, St. Louis, MO) were dissolved in saline and injected SC.

Data Analysis. Data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, $IS^{R}T$) and analyzed using a two-way repeated measures ANOVA with treatment condition and dose of cocaine as within subject factors. Data were analyzed using the SYSTAT statistical software package (Wilkinson *et al.*, 1992).

RESULTS

Acute pretreatment with the *direct* dopamine agonist APO (0.32, 1.0, or 3.2 mg/kg; Figure 1) failed to significantly affect the dose-response curve for cocaine self-administration. Similarly, acute pretreatment with the *indirect* dopamine agonists *d*-A (0.32, 1.0 and 3.2 mg/kg; Fig. 2) or METH (0.32, 1.0 and 3.2

mg/kg; Fig. 3) also failed to significantly affect the dose-response curve for cocaine self-administration. In contrast, one of the doses of *d*-A tested acutely, 1.8 mg/kg, produced a significant decrease in the rate of cocaine self-administration ($F_{(1,5)}$ =33.909; $p< 0.01$).

In contrast to results obtained in the dopamine agonist tests, the acute pretreatment with the two lowest doses of the *mixed* dopamine antagonist FLU (0.032 and 0.1 mg/kg) dose dependently shifted the cocaine self-administration curve to the right (Figure 4); (0.032, [$F_{(1,5)}$ = 20.552; $p< 0.05$]; 0.1, [$F_{(1,7)}$ = 42.93; $p< 0.001$). The highest dose of FLU tested; however, was toxic in 4 of the 7 subjects tested.

In addition, acute pretreatment with the *D1* dopamine antagonist SCH (0.0032, 0.01 and 0.032 mg/kg) dose dependently increased the rate of cocaine self-administration (Figure 5). When each dose was analyzed individually, all three doses tested significantly shifted the cocaine dose-response curve (0.0032, [$F_{(1,7)}$ = 18.54; $p< 0.01$]; 0.01, [$F_{(1,7)}$ = 139.621; $p= 0.001$]; 0.032, [$F_{(1,7)}$ = 13.719; $p= 0.01$]).

Similarly, acute treatment with the *D2* dopamine antagonist ETI (0.0032, 0.01 and 0.032 mg/kg) also dose dependently increased the rate of cocaine self-administration ([$F_{(3,18)}$ = 13.08; $p< 0.001$]; Figure 6). When the doses were analyzed individually, only the highest two doses of ETI (0.01 and 0.032 mg/kg)

tested significantly shifted the cocaine dose-response curve (0.01, [$F_{(1,6)}$ = 6.868; $p < 0.05$]; 0.032, [$F_{(1,6)}$ = 43.437; p= 0.001).

FIGURES

Figure 1

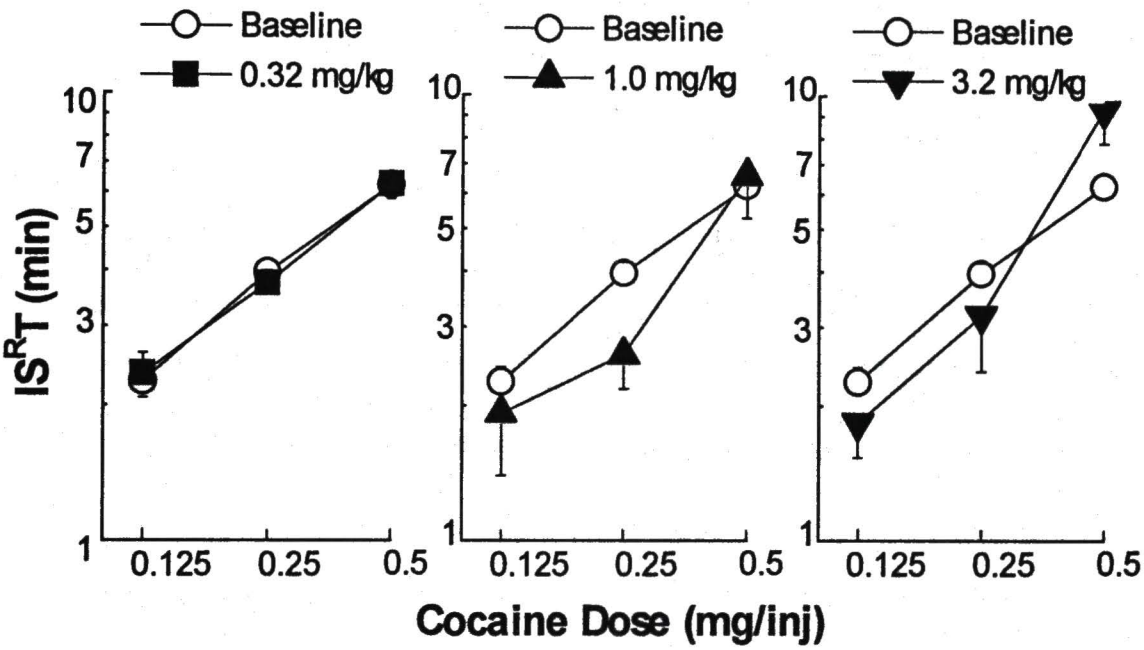

Fig. 1: The effects of acute treatment with APO (0.32, 0.1 and 3.2 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with APO. Data are represented as group means (n=6) ± S.E.M.

Figure 2.

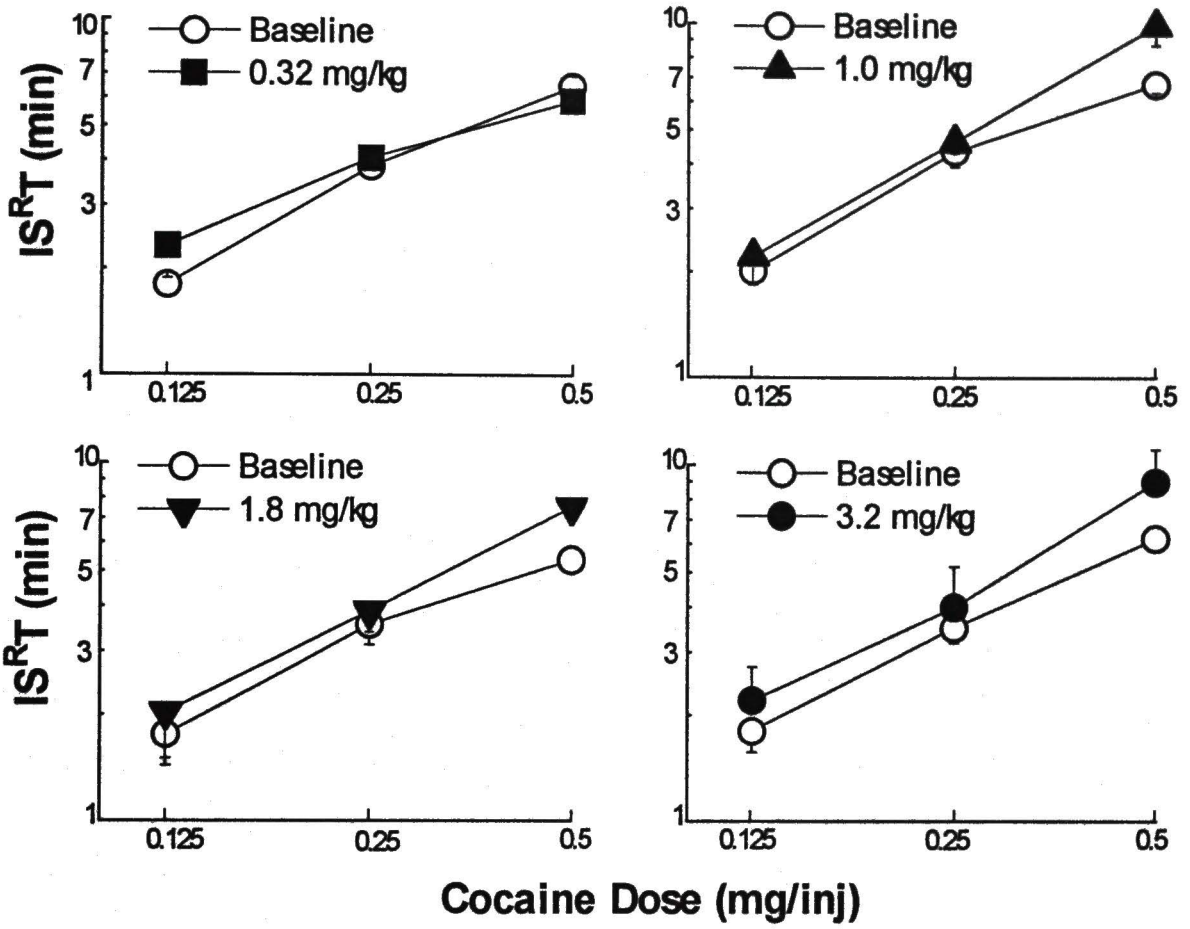

Fig. 2: The effects of acute treatment with *d*-A (0.32, 1.0, 1.8 and 3.2 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with *d*-A. Data are represented as group means (n=6) $\pm$ S.E.M.

Figure 3.

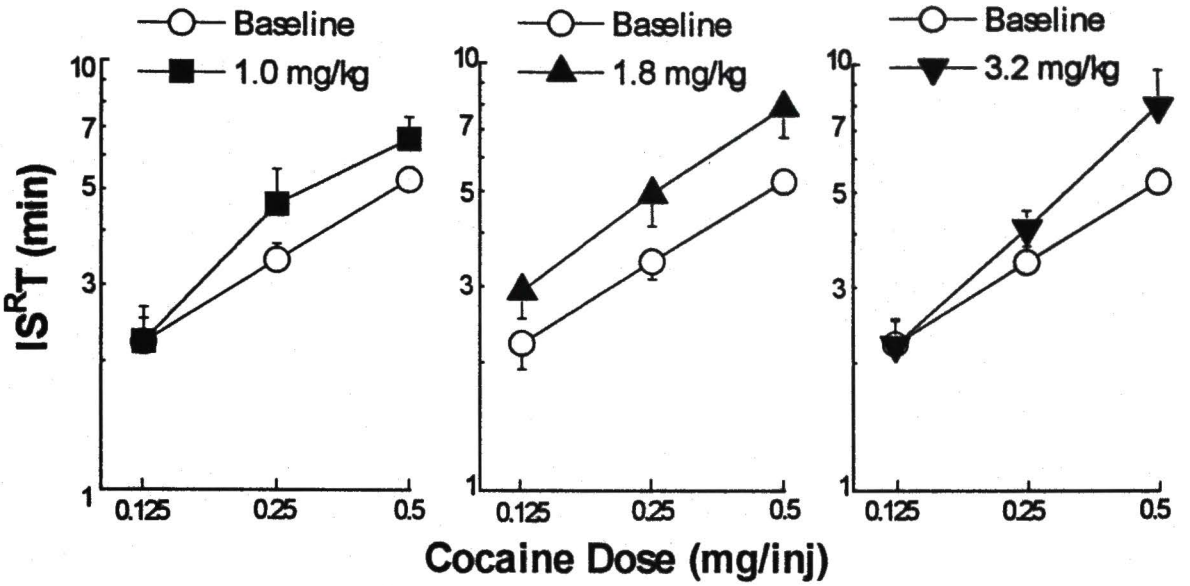

Fig. 3: The effects of acute treatment with METH (1.0, 1.8 and 3.2 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with METH. Data are represented as group means (n=6) $\pm$ S.E.M.

Figure 4.

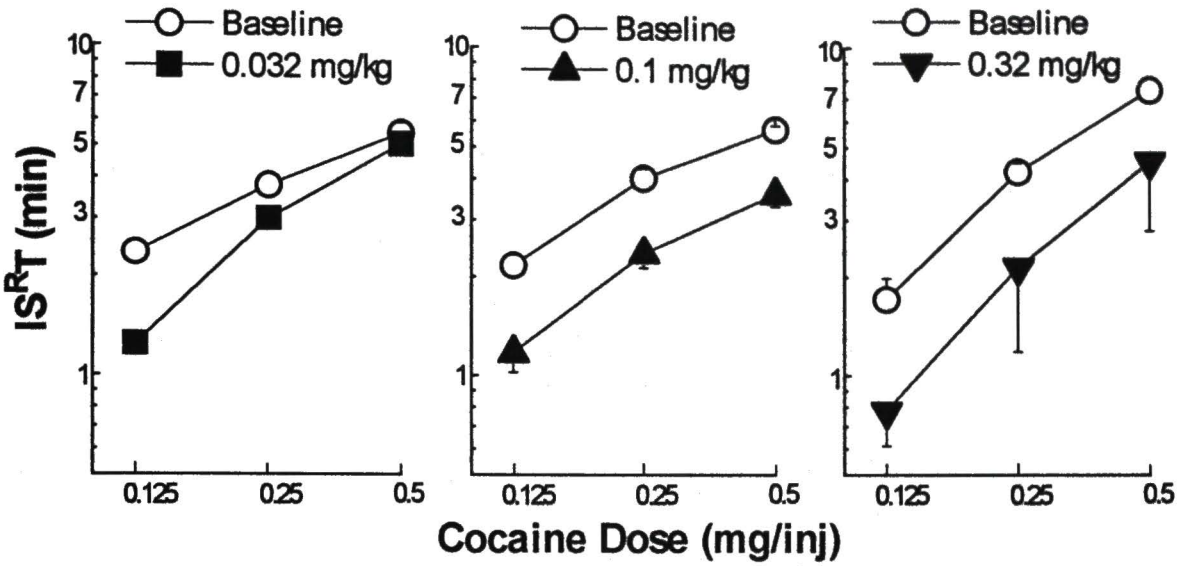

Fig. 4: The effects of acute treatment with FLU (0.032, 0.1 and 0.32 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with FLU. Data are represented as group means + S.E.M. N=6 except in the 0.32 mg/kg treatment group (n=3) due to toxicity.

Figure 5.

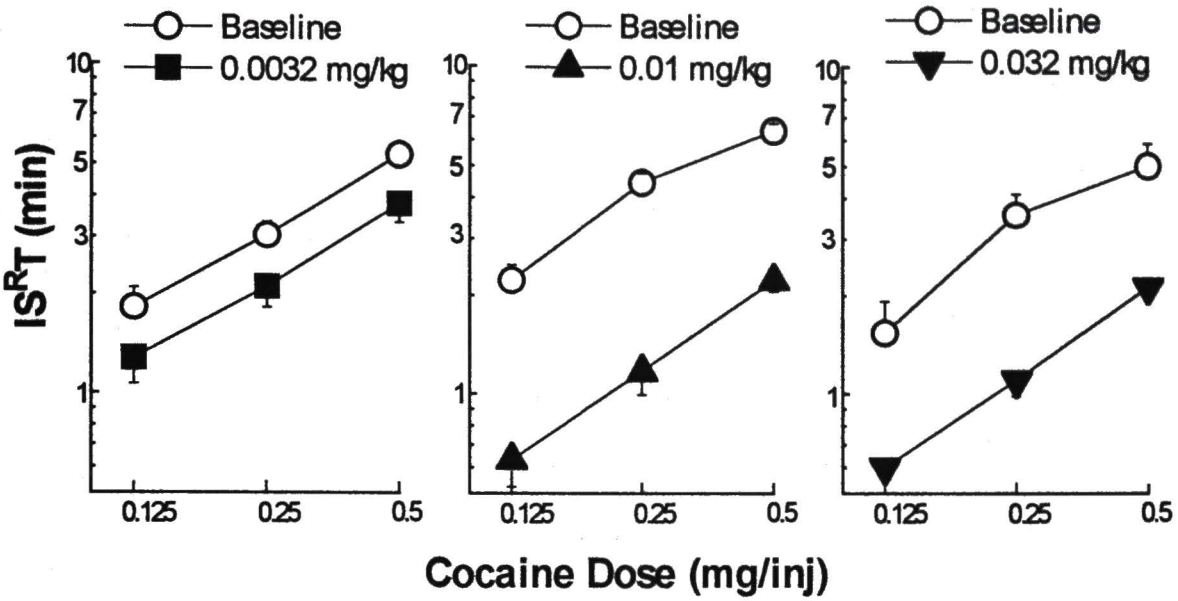

Fig. 5: The effects of acute treatment with SCH (0.0032, 0.01 and 0.032 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with SCH. Data are represented as group means (n=6) $\pm$ S.E.M.

Figure 6.

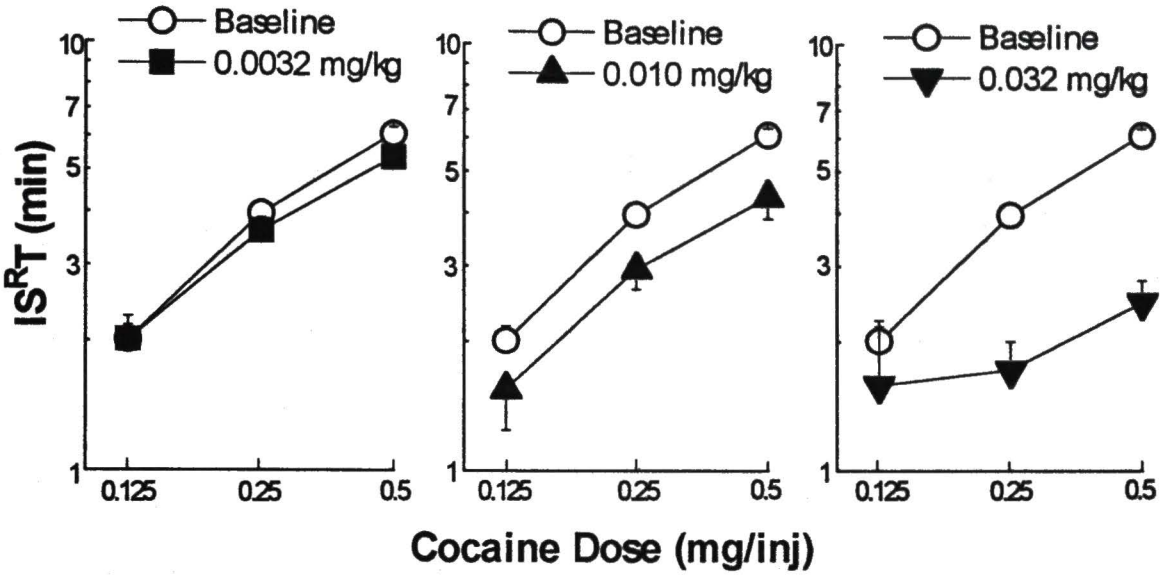

Fig. 6: The effects of acute treatment with ETI (0.0032, 0.01 and 0.032 mg/kg; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration an acute pretreatment with ETI. Data are represented as group means (n=6) $\pm$ S.E.M.

DISCUSSION

Acute pretreatment with the *direct* dopamine agonist apomorphine failed to alter the rate of cocaine self-administration. Similarly, with the exception of one dose of methamphetamine (1.8 mg/kg), acute pretreatment with the *indirect* dopamine agonists *d*-amphetamine and methamphetamine also failed to alter the rate of cocaine self-administration in rats. It is worth mentioning; however,

that acute pretreatment with these dopamine agonists produced behavioral intoxication that was manifested as a dose dependent increase in the delay of initiation of the self-administration behavior. There are other reports describing the effects of acute pretreatment with dopamine agonists on cocaine or *d*-amphetamine self-administration (Pickens *et al.*, 1968; Wilson and Schuster, 1973). These experiments demonstrated that acute pretreatment with *d*-amphetamine resulted in a decrease in the rate of either cocaine or *d*-amphetamine self-administration. The present results are consistent with those findings, in that pretreatment with 1.8 mg/kg of methamphetamine also decreased the rate of cocaine self-administration.

In contrast to the effects of acute pretreatment with dopamine agonists, the dopamine antagonists flupenthixol, SCH23390 and eticlopride all produced a dose dependent increase in the rate of cocaine self-administration, indicating the blockade of the reinforcing effects of cocaine. These data are in concordance with other reports in the literature demonstrate a blockade of the reinforcing effects of cocaine by dopamine antagonists (Britton *et al.*, 1991; Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Ettinberg *et al.*, 1982; Roberts and Vickers, 1984). In these reports, dopamine antagonists produce an increase in the rate of cocaine self-administration that is the same as that produced by a reduction of the unit dose of self-administered cocaine. This is similar to the present results, where pretreatment with the dopamine antagonists flupenthixol, SCH23390 and

eticlopride decreased the $IS^{R}Ts$ for cocaine self-administration, which is what occurs when the unit dose of cocaine is decreased. In summary, D1 and D2 dopamine antagonists increase the rate of cocaine self-administration, providing evidence that both of these receptor subtypes are important, but not required for the reinforcing effects of cocaine.

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CHAPTER 4

The experiments in this chapter were designed to test a portion of specific aim #7: To determine if the *chronic* administration of the direct DA *agonists* APO will result in *cross-tolerance* to the reinforcing effects of cocaine. The hypothesis is that the chronic administration of APO will result in a shift of the dose-response curve for cocaine self-administration to the right, indicating cross-tolerance to the reinforcing effects of cocaine. If stimulation of dopamine receptors by chronic cocaine treatment reduces the concentration or affinity of dopamine receptors to produce tolerance to cocaine's reinforcing effects, then chronic direct stimulation of these receptors by apomorphine may produce a similar change in receptor function and the reinforcing effects of cocaine.

Chronic treatment with the dopamine agonist apomorphine does not affect cocaine self-administration in rats[1]

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Abbreviations:
apomorphine (APO)
fixed ratio (FR)
injection (inj)
inter-reinforcer time (IS^RT)

ABSTRACT

This experiment was designed to test the hypothesis that the chronic administration of the direct dopamine (DA) agonist apomorphine (APO) would produce cross-tolerance to the reinforcing effects of cocaine. Rats (N=24) were implanted with indwelling jugular catheters and were trained to self-administer cocaine under a fixed-ratio 2 (FR2) schedule of reinforcement. After stable patterns of self-administration were observed, baseline dose-response curves were obtained. Rats were then treated chronically with APO (0, 0.32, 1.0 or 3.2 mg/kg/12 hr/7 days; SC). Twenty-four hours following the last chronic injection, dose-response curves for cocaine self-administration were again obtained. Chronic administration of APO, at any dose tested, failed to significantly shift the dose-response curve for cocaine self-administration. In summary, chronic treatment with APO does not produces cross-tolerance to the reinforcing effects of cocaine.

INTRODUCTION

Psychomotor stimulants like cocaine generally increase the availability of catacholamines (norepinephrine and dopamine) at central nervous system synapses by increasing release, decreasing reuptake, and/or inhibiting metabolism

(Weiner *et al.*, 1985). Dopamine, in particular, plays an important role in mediating the reinforcing effects of cocaine. For example, increased rates of cocaine self-administration are produced by pretreatment with various dopamine antagonists (Britton *et al.,* 1991; Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Ettinberg *et al.*, 1982; Roberts and Vickers, 1984). In contrast, acute pretreatment with the indirect dopamine agonist *d*-amphetamine results in a decrease in the rate of either cocaine or *d*-amphetamine self-administration (Pickens *et al.*, 1968; Wilson and Schuster, 1973).

An increase in response rate for cocaine self-administration has been interpreted as a reduction in the reinforcing properties of cocaine, because similar increases in response rate are also observed following reductions in the unit dose of cocaine (Caine and Koob, 1994; de Wit and Wise, 1977; Emmett-Oglesby *et al.*, 1993, Peltier *et al.*, 1996; Pickens and Thompson, 1968). In contrast, a decrease in response rate can be interpreted as an increase in the reinforcing properties of cocaine, because similar increases in response rate are also observed following increases in the unit dose of cocaine (Emmett-Oglesby *et al.*, 1993, Peltier *et al.*, 1996).

Chronic treatment with dopamine agonists has been shown to produce tolerance in drug self-administration paradigms in rats (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.,* 1993; Li *et al.*, 1994; McCown and Barrett, 1980). These reports have in common that they all tested indirect dopamine

agonists. The only report of the effects of chronic administration of direct dopamine agonists was an experiment that examined the effects of chronic apomorphine (APO) on cocaine discrimination (Wood and Emmett-Oglesby, 1987). Wood and Emmett-Oglesby (1987) treated rats chronically with APO (2.5 mg/kg/8 hr for 7 days). In that experiment, chronic APO shifted the dose-response curve for cocaine to the right, producing cross-tolerance to the discriminative stimulus effects of cocaine.

To date, the effects of chronic treatment with direct dopamine agonists on cocaine self-administration have not been investigated. Therefore, the aim of the present experiment is to determine the effects of chronically administered APO on cocaine self-administration in rats.

METHODS

Subjects. Subjects, male Fisher F-344 rats (n=24), were housed singly and maintained at 270 g $\pm$ 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training. For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were implanted with an indwelling catheter inserted into the right external jugular vein, the free end of which was fixed to the skull. After recovery, subjects were given the opportunity to self-administer cocaine on an FR1 schedule with a maximum of 25 cocaine infusions. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were then switched to an FR2 training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

The FR2 training schedule had a maximum of 15 reinforcers and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions.

Testing. Rats (n=18) were tested using a multi-dose procedure. With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/infusion) were available for self-administration during a single test session. This test session consisted of a standard priming infusion (0.3 mg) followed by 24 infusions. These 24 infusions were divided into three blocks of 8 infusions each, with the first block of eight reinforcers containing 0.5 mg/infusion of cocaine, the second

containing 0.25 mg/infusion and the third containing 0.125 mg/infusion. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/kg/infusion); testing was initiated with the high dose in order to increase the probability that the rats begin self-administration. Baseline dose-response curves for cocaine self-administration were obtained 24-hr before the chronic treatment. Twenty-four hours after the last chronic injection of APO, post-chronic dose-response curves for cocaine self-administration were obtained.

Chronic Treatment Regimen. After baseline dose-response data were obtained, the all training and testing were suspended. During this time, all rats received SC injections of APO (0, 0.32, 1.0 or 3.2 mg/kg/12 hr/7 days). Twenty-four hours after the last chronic injection of APO, post-chronic dose-response curves for cocaine self-administration were obtained.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 μm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. APO (Sigma, St. Louis, MO) was dissolved in water immediately before use, and injected SC.

Data Analysis. In FR2 self-administration experiments, data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, $IS^{R}T$) and analyzed using a two-way repeated

measures ANOVA with treatment condition and dose of cocaine as within subject factors. Data were analyzed using the SYSTAT statistical software package (Wilkinson *et al.*, 1992)

RESULTS

Increasing the dose of cocaine resulted in an orderly increase in the time between injections ([$F_{(2,10)}$ = 111.36; $p<0.001$]; Figure 1). Chronic treatment with vehicle resulted in a dose-response curve for cocaine self-administration that did not differ significantly from baseline. Similarly, chronic treatment with any dose of APO tested (0, 0.32, 1.0, or 3.2 mg/kg/12 hr for 7 days, SC; Figure 1) failed to significantly affect the dose-response curve for cocaine self-administration.

FIGURES

Figure 1.

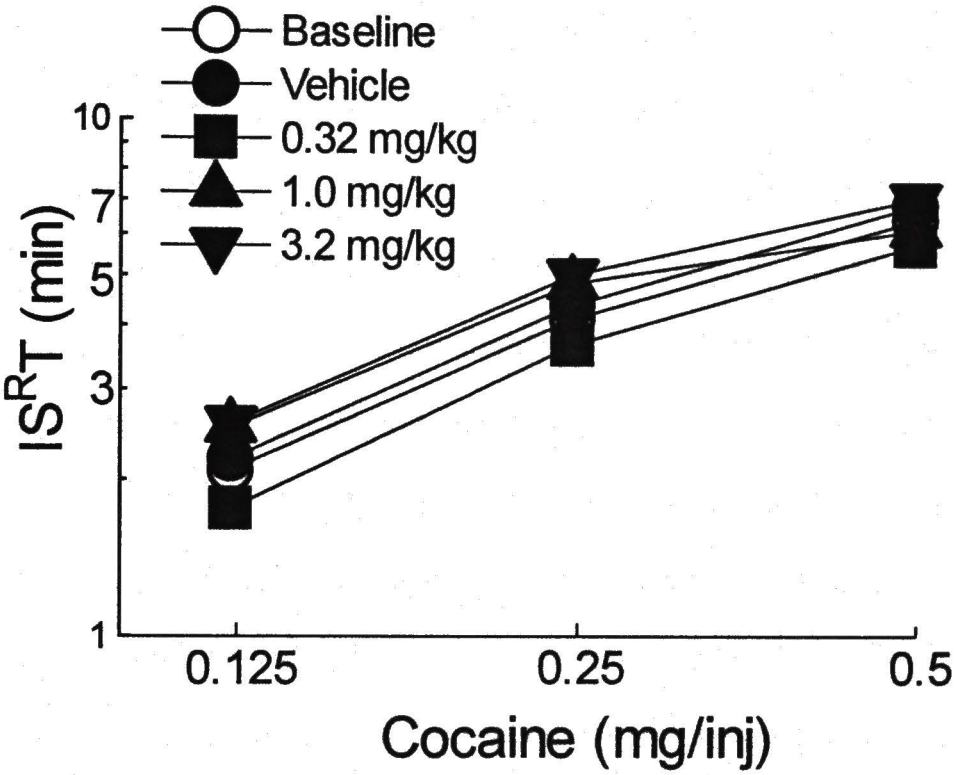

Fig.1: The effects of chronic treatment with APO (0.32, 1.0 or 3.2 mg/kg/12 hr for 5 days; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed symbols indicate the dose-response curve for cocaine self-administration 24 hours after the last chronic injection of APO. Data for the Baseline dose-response curve is represented as a group mean (n=24). Data for the post-chronic dose-response curves are represented as group means (n=6).

DISCUSSION

Chronic treatment with the direct dopamine agonist APO failed to significantly alter the dose-response curve for cocaine self-administration. These results are in contrast to the results obtained in a similar experimental paradigm, when rats were treated chronically with the indirect dopamine agonist cocaine (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). In both of the previous experiments, cocaine (20 mg/kg/8 hr/ 7 days; IV) treatment produced tolerance to the reinforcing effects of cocaine. The discrepancy in these findings may be due to the difference in the mechanism of action for cocaine and APO. Cocaine is an indirect dopamine agonists that acts by blocking the reuptake of dopamine (Taylor and Ho, 1978), which results in an increase in the amount of dopamine in the synapses. In contrast, APO is a direct dopamine agonist. It may be possible that long term stimulation of post-synaptic dopamine receptors by dopamine itself is required for the development of tolerance to the reinforcing effects of cocaine.

The current results are also in contrast to those obtained by Wood and Emmett-Oglesby (1987), who found that chronic treatment with APO produced cross-tolerance to the discriminative stimulus effects of cocaine. In that study, APO (2.5 mg/kg/8 hr/7 days) produced a two-fold shift to the right in the dose-response curve for cocaine discrimination. There are several possible reasons that the results obtained in that experiment differ from those obtained in this ex-

periment. The first reason, is that these two paradigms measure qualitatively different effects of cocaine. The drug discrimination paradigm measures the subjective effects of drugs, while the self-administration paradigm is a direct measure of the reinforcing properties of a drug. It is unlikely that this is the reason for the discrepancy in these results; however, because there has been very good concordance between results obtained in the drug discrimination and the drug self-administration procedures.

The second possible reason, is the different strains used in the two studies. In the drug discrimination study, Long-Evans rats were used, while in the present study, Fisher F-344 rats were used. It has been recently demonstrated that different strains of rats have different patterns of cocaine self-administration as well as differential responsiveness to acute treatment with dopamine antagonists (Ward *et al.*, 1996). The third possible reason is the difference in the dose of chronic APO. In the discrimination study, 2.5 mg/kg APO was injected every 8 hr for 7 days; while in the present study, 0.32, 1.0 or 3.2 mg/kg APO was injected SC every 12 hr for 7 days. It seems likely; however, that with the range of doses tested in the present experiment (0.32, 1.0 and 3.2 mg/kg), that any effects in the self-administration of cocaine produced by chronic APO would have been detected.

In summary, in contrast to the effects of chronic treatment with indirect dopamine agonists, chronic treatment with the direct dopamine agonists apomorphine failed to alter the reinforcing effects of cocaine in rats.

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CHAPTER 5

The experiments in this chapter were designed to test specific aim #7: To determine if the *chronic* administration of indirect DA *agonists* will result in *cross-tolerance* to the reinforcing effects of cocaine. The hypothesis is that the chronic administration of dopamine agonists will result in a shift of the dose-response curve for cocaine self-administration to the right, indicating cross-tolerance to the reinforcing effects of cocaine. This manuscript is published in *J. Pharmacol. Exp. Ther.* 277: 212-218, 1996.

Chronic *d*-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine[1]

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Abbreviations:

d-amphetamine (*d*-A)

methamphetamine (METH)

discriminative (S^D)

reinforcing (S^R)

fixed ratio (FR)

progressive ratio (PR)

injection (inj)

inter-reinforcer time (IS^RT)

ABSTRACT

These experiments tested the hypothesis that chronic administration of *d*-amphetamine (*d*-A) or methamphetamine (METH) would produce cross-tolerance to the discriminative (S^D) and/or reinforcing (S^R) effects of cocaine. One group of rats (N=20) was trained to detect cocaine (10.0 mg/kg; i.p.) from vehicle; cocaine (1.0-17.8 mg/kg) dose dependently substituted for the training dose. Chronic administration of *d*-A or METH (0.32, 1.0 and 3.2 mg/kg/12-hr for 7 days) resulted in cross-tolerance to the discriminative stimulus effects of cocaine. A second group of rats (N=12) was implanted with indwelling jugular catheters and were trained to self-administer cocaine under a fixed-ratio 2 (FR2) schedule of reinforcement. This group of rats also received chronic *d*-A or METH (0.32, 1.0 and 3.2 mg/kg/12-hr for 7 days). In this group, chronic administration of the highest dose of *d*-A and of METH (3.2 mg/kg) resulted in cross-tolerance to the self-administration of cocaine. A third group of rats (N=15) was implanted with indwelling jugular catheters and were trained to self-administer cocaine under a progressive-ratio (PR) schedule of reinforcement. Chronic administration of *d*-A and METH (3.2 mg/kg/12-hr for 7 days) resulted in cross-tolerance to the self-administration of cocaine under this PR schedule. The data obtained from these experiments demonstrate that chronic treatment with CNS stimulants of the amphetamine type (*d*-A or METH) produces cross-tolerance to both the S^D and S^R effects of cocaine.

INTRODUCTION

Tolerance develops to CNS stimulants in both drug discrimination (Barrett and Leith, 1981; Steigerwald *et al.*, 1994; Wood and Emmett-Oglesby, 1986, 1987, 1988, 1989; Young and Sannerud, 1989) and drug self-administration paradigms in rats (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993; Li et al., 1994; McCown and Barrett, 1980). For example, Wood and Emmett-Oglesby (1986, 1988) showed that in rats trained to discriminate cocaine, 10 mg/kg, from saline, a two-fold shift to the right of the cocaine dose-effect curve occurred after 7 days of chronic treatment with cocaine, either 10 or 20 mg/kg/8 hr. Using *d*-A as a training drug, Barrett and Leith (1981) showed that rats treated chronically with a total of 78 mg/kg of *d*-A over three days were tolerant to the training dose of *d*-A. Similarly, Steigerwald *et al.* (1994) showed that rats trained to discriminate *d*-A, 0.80 mg/kg, from saline demonstrated a three-fold shift to the right of the *d*-A dose-effect curve after chronic treatment with *d*-A, 3.2 mg/kg/12 hr for 7 days, and this tolerance increased to a four-fold shift after 14 days of treatment.

Tolerance to the S^R effects of cocaine has also been demonstrated in rats trained to self-administer cocaine under both low value FR (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993) and PR schedules (Li *et al.*, 1994). In parallel to the data obtained in the cocaine discrimination studies, rats trained to self-administer 15 injections of 1.0 mg/kg/inj of cocaine under an FR2

schedule of reinforcement demonstrated a two-fold shift to the right of the cocaine self-administration dose-response curve after receiving intravenous cocaine (20 mg/kg/8 hr for 7 days) (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). Similarly, rats trained to self-administer cocaine (0.9 mg/kg/injection) under a PR schedule, where an increasing number of responses is required to complete the ratio for each subsequent reinforcer, showed approximately two-fold tolerance to the reinforcing effects of cocaine after receiving intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days; Li *et al.*, 1994). In addition, tolerance to the S^R effects of *d*-A has also been demonstrated in rats trained to self-administer *d*-A under an FR1 schedule of reinforcement (McCown and Barrett, 1980). In that study, rats were trained to self-administer *d*-A, either 0.125 or 0.25 mg/kg/inj. Following chronic treatment with 78 mg/kg of *d*-A over three days, all subjects showed an increase in the amount of *d*-A self-administered by at least 45% over baseline levels.

To date, cocaine discrimination experiments have shown that chronic administration of a drug that substitutes for the cocaine training stimulus will result in cross-tolerance to the S^D effects of cocaine, while drugs that do not substitute for the cocaine training stimulus do not produce cross-tolerance to cocaine (Wood and Emmett-Oglesby, 1986, 1988). *d*-A administered in high doses to rats trained to discriminate cocaine (10 mg/kg) from saline, produced cross-tolerance to the discriminative stimulus properties of cocaine (Wood and Emmett-Oglesby,

1986); and chronic cocaine produced tolerance to the ability of CNS stimulants to substitute for cocaine (Wood and Emmett-Oglesby, 1988)

In summary, this lab has previously demonstrated that chronic high doses of cocaine produce tolerance to the S^D and S^R effects of cocaine. In addition, chronic administration of *d*-A produces cross-tolerance to the S^D effects of cocaine; similarly, chronic administration of cocaine produces cross-tolerance to the S^D effects of a wide variety of amphetamine-type compounds (Wood and Emmett-Oglesby, 1988). The present experiment was designed to confirm and extend these results by comparing the cross-tolerance effects of *d*-A and METH in 1) rats trained to discriminate cocaine and 2) rats trained to self-administer cocaine under either low value FR (FR2) or PR schedules of reinforcement. No previous studies have investigated cross-tolerance profiles for CNS stimulants in a self-administration paradigm. In addition, these studies provide information about whether S^D and S^R effects of cocaine are changed in parallel by chronic treatment with amphetamines; parallel changes would suggest that there is a common mechanism responsible for the development of tolerance to both the subjective and reinforcing effects of CNS stimulants. In contrast, if the S^D and S^R effects of cocaine are not changed in parallel by chronic treatment with amphetamines, it would suggest that there are separate mechanism responsible for the development of each type of tolerance.

METHODS

Cocaine Discrimination

Subjects. Twenty male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed singly and maintained at 330 ± 10 g by restricting their access to food. Water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training and Testing. Rats were trained to discriminate 10 mg/kg of cocaine (given in a volume of 1.0 ml/kg of 0.9% saline) from 0.9% saline under an FR10 two-lever drug discrimination paradigm with food as a reinforcer (45 mg pellets). The discrimination training sessions lasted until either 20 reinforcers were obtained or 20 min. elapsed. For the first 20 training sessions, rats were injected once daily 10 min before the beginning of the training sessions. Beginning on training session 21, rats received up to three injections per day, each followed by a training session. On days when multiple sessions were used in training, cocaine was always the last session of the day. No condition occurred in more than three successive training sessions. This procedure was adopted to ready animals for cumulative dose-effect testing.

Discriminative control was defined as 9 out of 10 successive sessions of correct lever responding at the start of the session; i.e. when cocaine was injected, 10 responses were emitted on the cocaine lever with fewer than 10 on the saline-lever; and when saline was injected, 10 responses were made on the saline-lever with fewer than 10 on the cocaine-lever. Once a criterion of nine out of ten consecutive correct-lever selections was met, cocaine dose-response tests were conducted. Cocaine discrimination was acquired in 40 training sessions (20 cocaine sessions and 20 saline sessions). Discrimination testing was conducted using a cumulative dosing method (Winger, 1980; Lane *et. al.,* 1992), which permits the determination of an entire dose-effect curve in a single session. This test procedure required approximately one-hr. For a more detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby (1990).

Self-administration

Subjects. For the FR2 and PR self-administration experiments, 12 and 15, respectively, male Fisher F-344 rats were housed singly and maintained at 270 $\pm$ 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training. For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were implanted with an indwelling catheter inserted into the right external jugular, the free end of which was fixed to the skull. After five days of recovery, subjects were given the opportunity to self-administer cocaine on an FR1 schedule with a maximum of 25 cocaine infusions. At the beginning of each training session, a priming injection of 0.3 mg of cocaine was given, with each subsequent injection consisting of 0.25 mg of cocaine in 0.1 ml. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were then switched to either an FR2 or PR training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

The FR2 training schedule had a maximum of 15 reinforcers (0.25 mg of cocaine, 0.1 ml) and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions. The PR training schedule began with a 0.30 mg/inj priming infusion of cocaine. To obtain each subsequent injection of the training dose (0.25 mg/inj), rats had to complete each of the ratios in the following sequence: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145,

178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, and 1347. There was a 1 hr time limit to obtain each reinforcer, and failure to do so terminated the session. The last reinforcer that was obtained for each session was termed the breaking point. Rats were tested once they met a criterion of the total number of reinforcers obtained in one session (breaking point) not varying by more than three injections across seven consecutive training sessions.

FR2 self-administration testing. Rats (n=12) were tested using a multi-dose procedure (Emmett-Oglesby *et al.*, 1993). With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/infusion) were available for self-administration during a single test session. This test session consisted of a priming infusion (0.3 mg) followed by 24 infusions. These 24 infusions were divided into three blocks of 8 infusions each, with the first block of eight reinforcers containing 0.5 mg/infusion of cocaine, the second containing 0.25 mg/infusion and the third containing 0.125 mg/infusion. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/kg/infusion); testing was initiated with the high dose in order to increase the probability that the rats would begin self-administration. Rats were tested both 24-hr before, and 24-hr following the last chronic injection of *d*-A or METH. All subjects were tested under each chronic condition (described below) in a random order.

PR self-administration testing. After the stability criterion was met, rats (n=15) were tested by substituting different doses of cocaine (0.028 and 0.083 mg/inj) for the training dose (0.25 mg/inj). These substitutions took place several times until reproducible dose-response data were obtained. Subsequently, for the pre-chronic phase of these experiments these same doses of cocaine were tested, one dose per day, on the three days immediately prior to the start of the chronic injections. The order of dose presentation was random across the group; however, the same order was used for each rat throughout all tests. In all tests, subjects were first infused with the dose of cocaine available for testing on that day. Once the pre-chronic dose-response curves were obtained, rats were randomly assigned to one of two test groups; *d*-A (n=7) and METH (n=8). The post-chronic dose-response curve was obtained by making the same dose substitutions on the three days immediately following the chronic injections, with the first substitution occurring 24 hr after the last chronic injection of *d*-A or METH. After the post-chronic dose-response curve was obtained, the rats were allowed one week without testing or training, after which time, a dose-response curve was again obtained (this was termed the recovery curve).

Chronic Treatment with d-A or METH.

After baseline dose-response data were obtained in both the cocaine discrimination and the cocaine self-administration experiments, all training and testing were suspended. During this time, all rats in the drug discrimination and FR2 self-administration procedures received injections of either d-A or METH (0.32, 1.0, and 3.2 mg/kg, s.c.), once every 12 hr for seven days. In the PR procedure, only a single dose of either d-A or METH (3.2 mg/kg, s.c., every 12 hr for seven days) was examined. Tests were limited to this dose for two reasons: it was the only effective dose in the other two paradigms, and the duration required to train and stabilize dose-effect testing in the PR paradigm was lengthy enough that it was unlikely that subjects would maintain viable catheters if more than one dose was tested chronically. Twenty-four hours following the last chronic injection, cocaine dose-response curves were reobtained. In the discrimination and FR2 experiments, following the post-chronic dose-response curve tests, rats were allowed one week without testing or training to recover from the chronic experiment, after which time, another dose-response curve was obtained. This curve was termed the recovery curve. Rats were then trained until their baseline IS^RT's were within 20% of pre-chronic values, which took approximately one week. When the baseline IS^RT's were stable, they were then included in another chronic regimen using a different dose of either *d*-A or METH. Subjects were assigned to receive either *d*-A or METH; within this as-

signment, all subjects received all doses in a random block design. Thus, at each round of chronic administration, all three doses were assigned across subjects, and across repetitions of the experiment, these doses were shifted such that all subjects received all doses.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in 0.9% saline for drug discrimination experiments and injected i.p. For self-administration experiments, cocaine HCl was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 µm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. *d*-A sulfate (Sigma, St. Louis, MO) and METH HCl (Sigma, St. Louis, MO) were dissolved in 0.9% saline and injected s.c.

Data Analysis. In the cocaine discrimination experiment, data were scored and analyzed in terms of the percent of total responses emitted upon the cocaine-appropriate lever and as the rate at which rats responded upon both levers (responses per second). Full cocaine-lever selection was defined as 80% or more of the responses occurring on the cocaine-appropriate lever. ED_{50} values were calculated as an estimate for each individual curve designating the maximum cocaine-lever responding for that curve as a 100% value. ED_{50} values and their 95% confidence limits were calculated using Pharmacological Calculation Systems software for the IBM computer.

To determine if dose-effect curves were significantly different from one another, the data was transformed using logit transformation (log [x/100-x]; where X is the percentage of responses emitted on the drug-appropriate lever for each subject). When a subject responded solely upon one of the two levers, resulting in either 0% or 100% drug-lever responding, these data were first transformed into either 1% or 99% drug-lever responding, respectively, prior to the logit transformation. The logit transformation accentuates the differences at the two ends of the data range such that the distribution of the percentage data becomes more normal. The transformed data were subjected to a between groups analysis of variance (ANOVA) to see if there was an effect of the chronic treatment (SAS for the microcomputer). Degrees of freedom for these tests were based upon within subjects design.

In FR2 self-administration experiments, data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, IS^RT) without including the 30s time-out that followed the delivery of each reinforcer. This measure is the reciprocal of reinforcers per unit time. For training sessions, the time between the start of the session and self-administration of the first reinforcer was not included in the data analysis because this time was more variable than subsequent IS^RTs. Thus, for the multi-dose test procedure, only the last 7 IS^RTs for each dose of cocaine were used for data analysis. A subject was required to take all available reinforcers during

the training or testing sessions to be included in the analysis. In addition, the dependent variable is shown on a log scale because this transformation readily shows the uniform effect of chronic treatment regimens across the entire dose-response curve. Data were analyzed using a 2 x 3 x 3 way repeated measures ANOVA with treatment condition, dose of either *d*-A or METH, and dose of cocaine as within subject factors.

In PR self-administration experiments, the number of reinforcers obtained was used as the dependent measure, which is termed the breaking point. This breaking point measure was used rather than the final ratio completed or the total number of responses emitted because these latter variables are not amenable to parametric analysis (for a discussion of this problem in analysis of response and reinforcer data from PR procedures, see Depoortere *et. al.*, 1993; and Roberts and Richardson, 1992). Breaking points were analyzed using a two-way repeated measures ANOVA with treatment condition and dose of cocaine as within subject factors. SYSTAT software (Wilkinson *et. al*, 1992) was used for data analysis.

RESULTS

Cocaine Discrimination: *Effect of chronic* d-*A and METH on the discrimination of cocaine.* In subjects trained to detect cocaine (10.0 mg/kg), using a cumulative dose testing procedure (1.0, 3.2, 10.0 and 17.8 mg/kg of cocaine), cocaine substituted for itself in a dose-dependent manner with full substitution occurring with 17.8 mg/kg of cocaine (Figures 1 & 2). ED50 values with associated 95% confidence limits for cocaine discrimination data are shown in Table 1. Prior to chronic *d*-A or METH treatment, these values for the two groups were 3.25 and 3.84 mg/kg, respectively. Chronic treatment with *d*-A (0.32, 1.0 and 3.2 mg/kg/12 hr for 7 days, s.c.) increased the ED_{50} for the cocaine discrimination curve without affecting the response rate (Table 1; Figure 1; graphs A and B, respectively). Chronic injections of the two lowest doses of *d*-A (0.32, 1.0 mg/kg) increased the ED_{50} between 1- to 2-fold, while injections with the highest dose of *d*-A increased the ED_{50} approximately 4- to 5-fold ($F_{(1,8)}$ =26.55, $p < 0.001$). Chronic treatment with METH (0.32, 1.0 and 3.2 mg/kg/12 hr for 7 days, s.c.) dose-dependently increased the ED_{50} for the cocaine discrimination curve, also without significantly effecting the response rate (Table 1; Figure 2; graphs A and B, respectively). The lowest dose of chronic METH (0.32 mg/kg) did not significantly change the ED_{50}, the intermediate dose of chronic METH (1.0 mg/kg) increased the ED_{50} approximately 3-fold ($F_{(1,8)}$ = 10.34, $p < 0.025$) and the highest

dose of chronic METH (3.2 mg/kg) increased the ED_{50} 4-fold ($F_{(1,9)} = 16.55$, $p < 0.005$).

Self-Administration: *Effect of chronic* d-A *and METH on the rate of cocaine self-administration under an FR2 paradigm.* Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the time between injections ([$F_{(2,10)} = 168.2$; $p < .001$]; [$F_{(2,10)} = 189.8$; $p < .001$]) (Figure 3). Chronic treatment with *d*-A (0.32, 1.0 and 3.2 mg/kg/12 hr for 7 days, s.c.; Figure 3) shifted the cocaine self-administration curve significantly to the right ($F_{(2,10)} = 4.3$; $p < .05$). When each dose was analyzed independently, only the highest dose of *d*-A given chronically (3.2 mg/kg) resulted in a significant shift of the cocaine dose-response curve ($F_{(1,5)} = 7.7$; $p < .05$). Similarly, only the highest dose of METH given chronically (3.2 mg/kg) resulted in a significant shift of the cocaine dose-response curve ($F_{(1,5)} = 21.6$; $p < .01$). Following one week of recovery from the chronic treatment of either *d*-A or METH, the dose-response curves for cocaine self-administration returned to baseline levels of self-administration (Table 2).

Effect of chronic d-A *and METH on cocaine self-administration under a PR paradigm.* Under baseline conditions, increasing the dose of cocaine resulted in an orderly increase in the breaking point ([$F_{(2,12)} = 19.5$; $p < .001$]; [$F_{(2,14)} = 96.6$; $p < .001$]) (Figure 4). Chronic treatment with *d*-A (3.2 mg/kg/12 hr for 7 days, s.c.; Figure 4) shifted the dose-response curve for breaking points significantly

to the right ($F_{(1,6)} = 22.3$; $p < .01$). In addition, following one week of recovery from chronic *d*-A injections, the dose-response curve for cocaine self-administration spontaneously returned to pre-chronic levels (Fig 4). Chronic METH treatment (3.2 mg/kg/12 hr for 7 days, s.c.; Figure 4) also shifted the dose-response curve for cocaine self-administration significantly to the right ($F_{(1,7)} = 26.7$; $p = .001$). Similar to the *d*-A group, one week of recovery from chronic METH injections produced a spontaneous return to pre-chronic levels of cocaine self-administration (Fig 4).

TABLES

Table 1. Comparison between baseline and post-chronic ED_{50}s and 95% confidence limits for rats trained to discriminate cocaine, 10 mg/kg, from saline.

d-amphetamine

	ED50	95% C.L.
Baseline	3.25	2.18 - 4.85
Post-0.32 mg/kg	5.47	2.25 - 13.3
Post-1.0 mg/kg	4.89	2.42 - 9.88
Post-3.2 mg/kg	*14.83	8.28 - 26.59

methamphetamine

	ED50	95% C.L.
Baseline	3.84	2.58 - 5.27
Post-0.32 mg/kg	4.74	2.22 - 10.16
Post-1.0 mg/kg	*12.47	6.14 - 25.33
Post-3.2 mg/kg	*16.67	9.47 - 29.34

Table 2. Comparison between pre-chronic baseline dose-response curves and post-chronic recovery dose-response curves for rats self-administering cocaine under an FR2 schedule. Data are presented as time between reinforcers (IS^RT's in minutes) ± S.E.M.

d-amphetamine (n=6)

Cocaine Dose (mg/kg/inf)	Pre-chronic Baseline	Recovery		
		0.32 mg/kg	1.0 mg/kg	3.2 mg/kg
0.125	1.89 ± 0.14	1.85 ± 0.12	1.61 ± 0.09	1.69 ± 0.37
0.25	4.35 ± 0.35	4.46 ± 0.45	3.94 ± 0.40	4.18 ± 0.54
0.5	6.90 ± 0.41	6.88 ± 0.51	6.39 ± 0.24	6.02 ± 0.64

methamphetamine (n=6)

Cocaine Dose (mg/kg/inf)	Pre-chronic Baseline	Recovery		
		0.32 mg/kg	1.0 mg/kg	3.2 mg/kg
0.125	2.04 ± 0.12	1.98 ± 0.15	1.76 ± 0.10	2.16 ± 0.35
0.25	4.58 ± 0.23	4.90 ± 0.26	4.41 ± 0.22	4.24 ± 0.40
0.5	6.98 ± 0.31	6.85 ± 0.36	6.47 ± 0.48	6.87 ± 0.27

FIGURES

Figure 1.

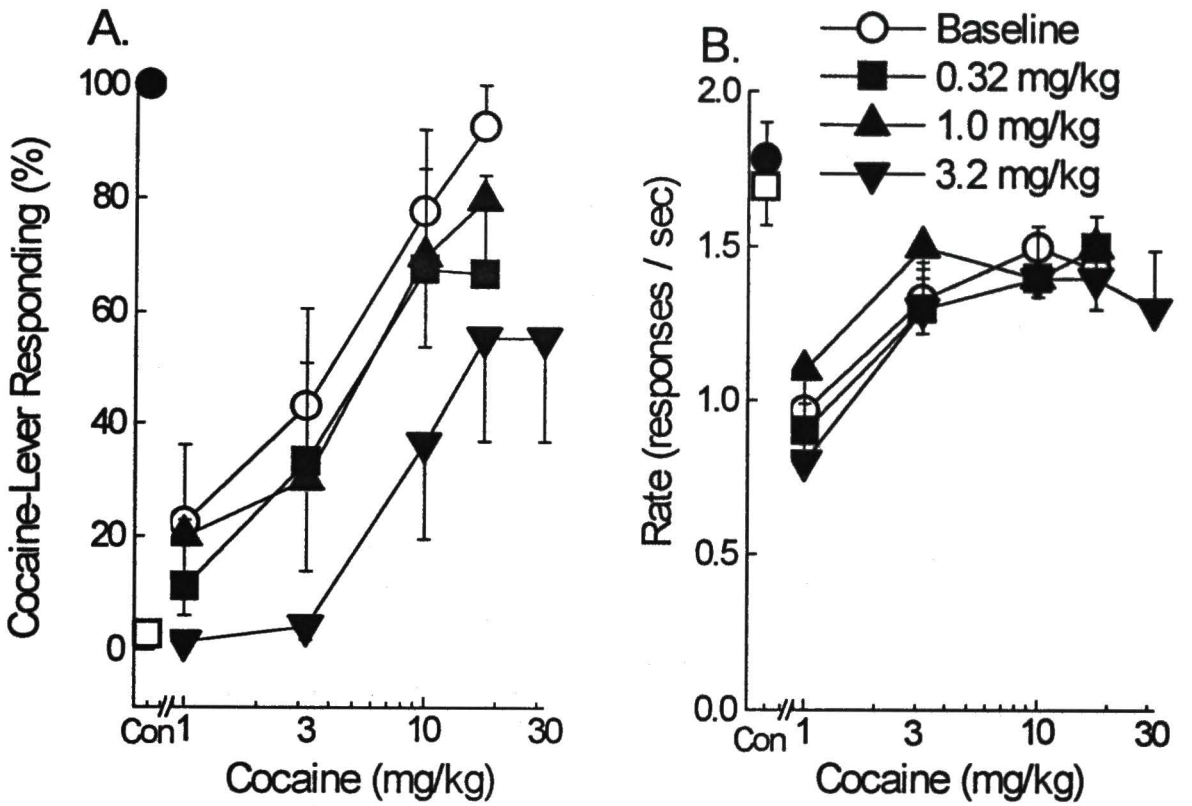

Fig. 1. Effect of chronic *d*-A (0.32, 1.0 and 3.2 mg/kg; s.c.) on the discriminative stimulus produced by cocaine. Abscissa: Dose of cocaine obtained in a cumulative dosing procedure. Subjects were tested repeatedly with increasing doses of cocaine as shown on the abscissa. Ordinate: Graph A, percent of responding on the cocaine lever; Graph B, rate of lever presses for both levers. Closed circles (●) indicate percent lever responding (Graph A) and response rate (Graph B) at the training dose of cocaine (10.0 mg/kg) and open squares (□) indicate percent lever responding (Graph A) or response rate (Graph B) for saline. Open circles (O) indicate baseline dose-effect data (N=11); closed squares (■) indicate chronic treatment with *d*-A (0.32

mg/kg/12 hr/7 days); up triangles (▲) indicate chronic treatment with *d*-A (1.0 mg/kg/12 hr/7 days); down triangles (▼) indicate chronic treatment with *d*-A (3.2 mg/kg/12 hr/7 days). Data are presented as means ± S.E.M.

Figure 2.

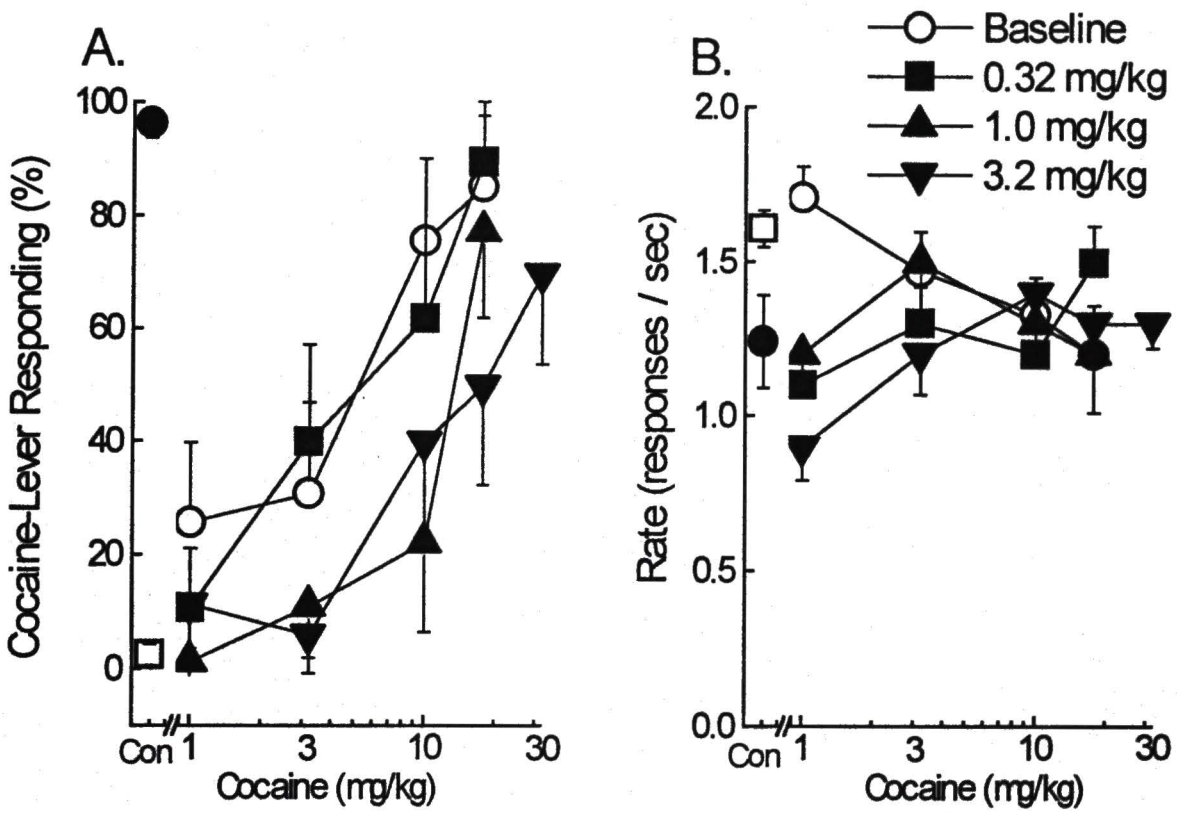

Fig. 2. Effect of chronic METH (0.32, 1.0 and 3.2 mg/kg; s.c.) on the discriminative stimulus produced by cocaine. Abscissa: Dose of cocaine obtained in a cumulative dosing procedure. Subjects were tested repeatedly with increasing doses of cocaine as shown on the abscissa. Ordinate: Graph A, percent of responding on the cocaine lever; Graph B, rate of lever presses for both levers. Closed circles (●) indicate percent lever responding (Graph A) and re-

sponse rate (Graph B) at the training dose of cocaine (10.0 mg/kg) and open squares (□) indicate percent lever responding (Graph A) or response rate (Graph B) for saline. Open circles (O) indicate baseline dose-effect data (N=11); closed squares (■) indicate chronic treatment with METH(0.32 mg/kg/12 hr/7 days); up triangles (▲) indicate chronic treatment with METH (1.0 mg/kg/12 hr/7 days); down triangles (▼) indicate chronic treatment with METH (3.2 mg/kg/12 hr/7 days). Data are presented as means ± S.E.M.

Figure 3.

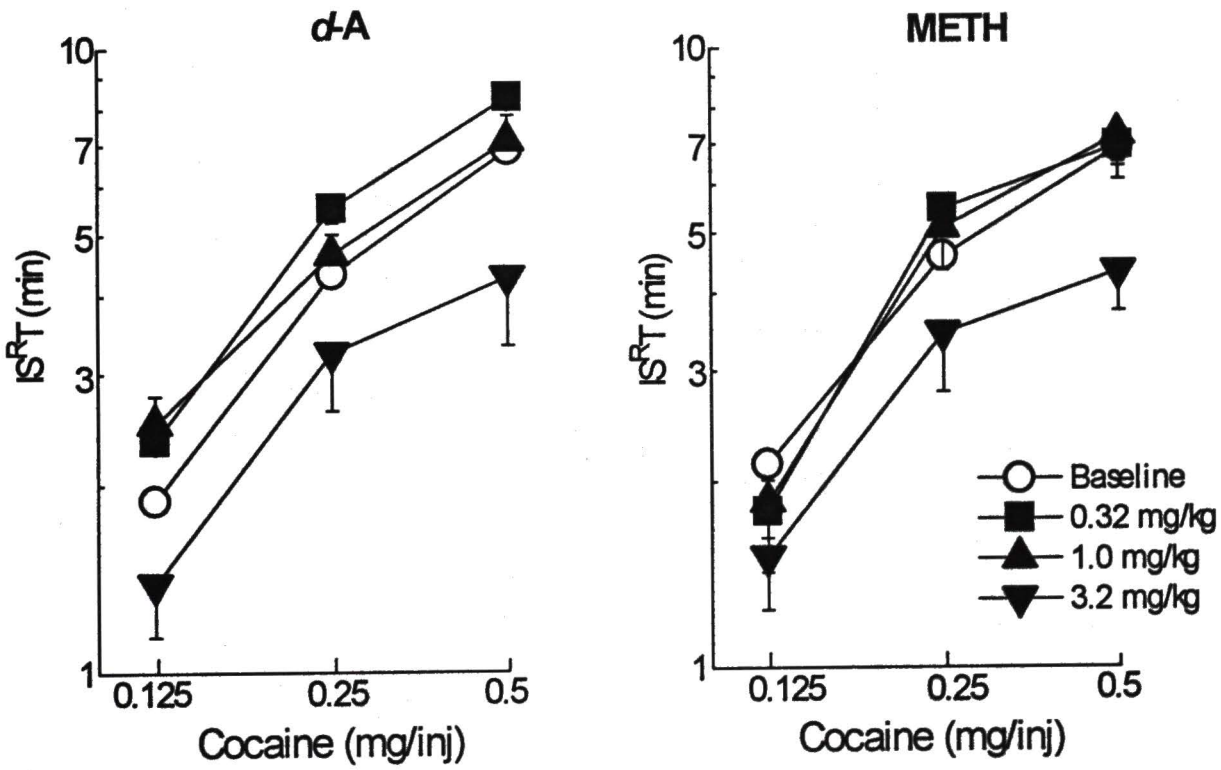

Fig. 3. Effect of *d*-A or METH on cocaine self-administration under a FR2 paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: inter-reinforcer time (min.). Open circles (O) indicate self-administration of cocaine prior to chronic treatment; closed

symbols indicate self-administration of cocaine 24 hr following the last chronic injection of *d*-A (top panel) or METH (bottom panel). Squares (■) indicate chronic treatment with (0.32 mg/kg/12 hr/7 days); up triangles (▲) indicate chronic treatment with (1.0 mg/kg/12 hr/7 days); down triangles (▼) indicate chronic treatment with (3.2 mg/kg/12 hr/7 days). The same subjects (N=6) received all treatments in a randomized order. Data are shown as mean ± S.E.M.

Figure 4.

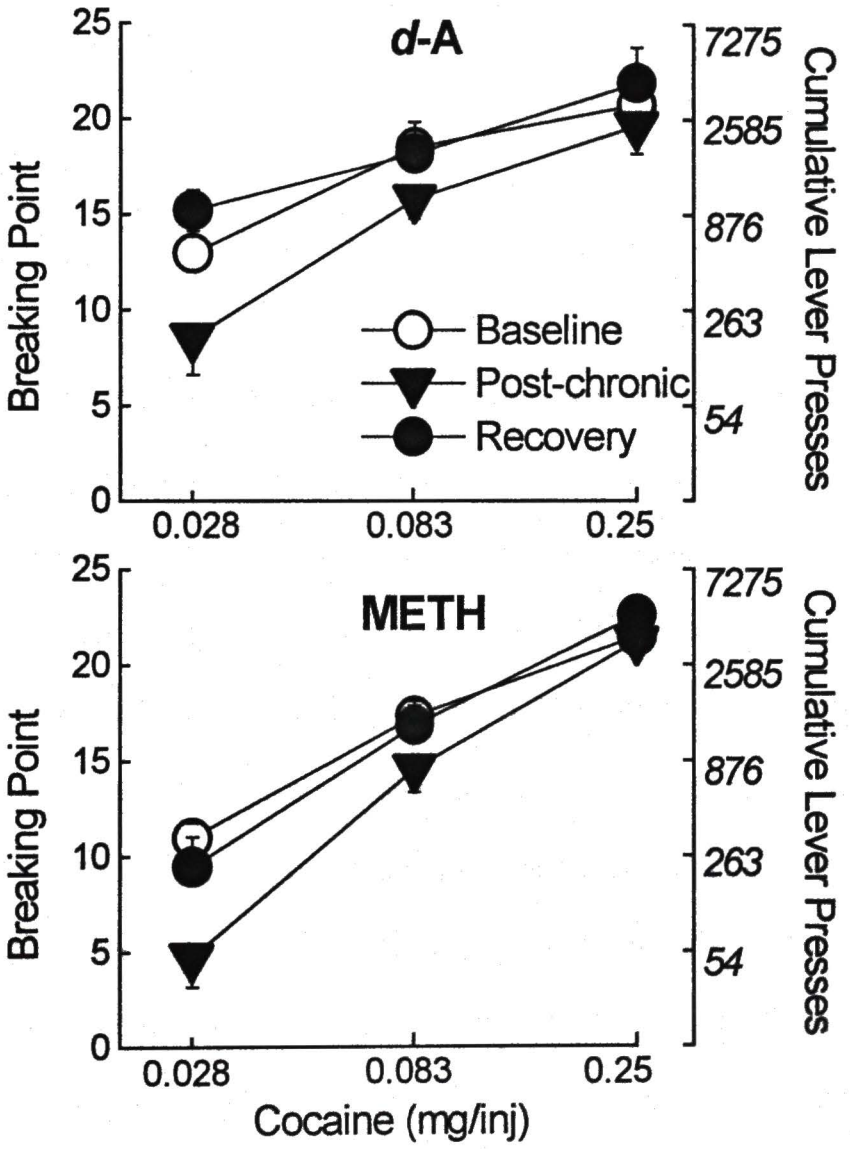

Fig. 4. Effect of *d*-A or METH on cocaine self-administration under a PR paradigm. Abscissa: Dose of cocaine made available for self-administration. Ordinate: breaking point. Open circles (O) indicate self-administration of cocaine prior to chronic treatment. Down triangles (▼) indicate chronic treatment with *d*-A (top panel) or METH (bottom panel) (3.2 mg/kg/12 hr/7 days); closed circles (●) indicate dose-response data obtained 10 days following the last chronic injection of *d*-A or METH. Data are shown as mean ± S.E.M.

DISCUSSION

In drug discrimination experiments, prior to chronic *d*-A or METH administration doses of cocaine higher than the training dose were required to obtain full substitution. Although we cannot specify why an increase in dose is necessary to obtain substitution, two possibilities seem most likely. First, the cumulative dosing procedure required approximately 60 minutes from start to finish. Cocaine has a very brief half-life in rats (less than 20 minutes; Nayak *et al.*, 1976); thus, the doses reported in the cumulative procedure may underestimate actual blood concentrations achieved using this method. Alternately, in humans, tolerance has been reported to occur to the subjective effects of cocaine within a session of use (Fischman *et al.*, 1985; Ambre *et al.*, 1988), and it may be the case that some degree of within session tolerance accounts for the necessity of

going to higher doses than the training dose. In either case, animals show stimulus control and full substitution to the 80% training criterion.

Chronic treatment with *d*-A produced cross-tolerance to cocaine in the discrimination procedure. The two lowest doses of *d*-A given chronically (0.32 and 1.0 mg/kg) had similar effects, producing between a one- to two-fold shift to the right in the dose-response curve for cocaine discrimination, with the highest dose of chronic *d*-A (3.2 mg/kg) producing an approximate four- to five-fold shift to the right. These findings are similar to previous reports that chronic treatment with *d*-A (2.5 mg/kg/8 hr for 7 to 9 days) produced a four-fold shift to the right of the dose-response curve for cocaine discrimination (Wood and Emmett-Oglesby, 1986). Similar to the affects of chronic *d*-A, chronic treatment with METH produced dose-dependent cross-tolerance to cocaine in the discrimination procedure. The chronic treatment with *d*-A or METH, at any dose tested, did not effect the response rate for cocaine discrimination.

Chronic treatment with *d*-A produced cross-tolerance to cocaine under an FR2 schedule of cocaine self-administration. These results were slightly different from those observed in the cocaine discrimination tests, in that the two lowest doses of *d*-A given chronically, 0.32 and 1.0 mg/kg, did not significantly alter the dose-response curve for cocaine self-administration. It is not clear whether this result is the consequence of using two different strains of rats, or whether it reflects fundamental differences in paradigms. For example in drug discrimination,

subjects are trained with a fixed dose of drug, whereas in self-administration under the FR2 schedule, degree of drug-effect is controlled by the subject.

In contrast to the lower doses of *d*-A, the highest dose of *d*-A tested, 3.2 mg/kg, shifted the dose-response curve for cocaine self-administration approximately two-fold to the right. The degree of cross-tolerance produced by *d*-A (3.2 mg/kg/8 hr) in this study was similar to the degree of tolerance produced by cocaine (20 mg/kg/8 hr) under a similar experimental protocol (Emmett-Oglesby *et al.*, 1993).

Chronic treatment with METH also produced cross-tolerance to cocaine under an FR2 schedule of cocaine self-administration. These results were similar to those obtained with chronic *d*-A treatment in that the two lowest doses of METH given chronically, 0.32 and 1.0 mg/kg, did not significantly alter the dose-response curve for cocaine self-administration (these doses also resembled chronic saline treatment). However, the highest dose of METH tested, 3.2 mg/kg, did shift the dose-response curve for cocaine self-administration approximately two-fold to the right.

It is apparent from the data obtained in this experiment that the two lowest doses of *d*-A and METH tested (0.32 and 1.0 mg/kg) were not large enough to produce cross-tolerance to cocaine in the FR2 self-administration paradigm. Similar to the data obtained in the FR2 self-administration experiment, only the

highest dose of chronic *d*-A (3.2 mg/kg) produced cross-tolerance to the discriminative stimulus affects of cocaine. In contrast, the two highest doses of chronic METH, 1.0 and 3.2 mg/kg, produced cross-tolerance to cocaine in the discrimination experiment. It is also worth noting that the 3.2 mg/kg dose of either *d*-A or METH produced a much larger shift in the dose-response curve for cocaine discrimination than for cocaine self-administration (4-fold and 2-fold respectively). One possible reason for these results is the different strains of rats that were used. Sprague-Dawley rats were used in the discrimination experiments and Fisher F-344 rats were used in the self-administration experiments.

Based on the results of the FR2 self-administration experiments, only the highest dose of *d*-A (3.2 mg/kg) and METH (3.2 mg/kg) were examined using a PR self-administration paradigm. Chronic treatment with this dose produced cross-tolerance to cocaine under a PR schedule of cocaine self-administration. Chronic treatment with either *d*-A or METH resulted in an approximate two-fold shift of the cocaine dose-response curve to the right. Although there is a significant decrease in breaking points, it is only a decrease of 1 to 6 reinforcers. However, the decrease in the real number of lever presses ranges from approximately 300 to 800 responses. These data were similar to data obtained when rats received intravenous cocaine (20 infusions of 0.9 mg/kg/8 hr for 7 days) (Li *et al.*, 1994). In that study, chronic treatment with cocaine produced approximately two-fold tolerance to the reinforcing effects of cocaine as demon-

strated by a shift to the right of the cocaine self-administration dose-response curve.

Although chronic treatment with either *d*-A or METH produced an increase in the number of responses in the FR2 paradigm and a decrease in the number of responses in the PR paradigm, both results are indicative of tolerance. If a rat is tolerant to the S^R effects of cocaine in an FR2 or PR paradigm, a given dose of cocaine will now be self-administered as if it were a lower dose. In these experiments, doses of cocaine were used that were on the descending limb of the dose-effect curve in the FR2 paradigm. On the descending limb of the dose-response curve, there is an inverse relationship between the dose of cocaine available for self-administration and the number of reinforcers obtained. In other words, as the dose of cocaine decreases, rats will respond faster. In contrast, in the PR paradigm there is a direct relationship between the dose of cocaine available for self-administration and the number of responses that a subject emits. If a rat is tolerant to the S^R effects of cocaine, then fewer reinforcers should be obtained under the PR schedule.

S^D and S^R effects of cocaine are mediated at least in part by mesolimbic dopamine systems (Koob and Bloom, 1988; Di Chiara, 1995; Wood and Emmett-Oglesby, 1989). Direct electrical stimulation of these dopamine neurons will serve as a reinforcer, and CNS stimulants are known to potentiate the reinforcing effects of electrical brain stimulation. Chronic treatment with *d*-A (Leith and

Barrett, 1981; Wise and Munn, 1985) or cocaine (Markou and Koob, 1991) produces an increase in brain self-stimulation reward thresholds. These data have been interrupted as providing evidence for cocaine-withdrawal-induced "anhedonia" (Markou and Koob, 1991); however, the data from self-stimulation experiments can also be interrupted as showing tolerance to the reinforcing effects of the stimulating current (larger current intensities are required to produce a reinforcing effect). It may be the case that withdrawal from chronic cocaine is in fact the phenomenon giving rise to tolerance in all of these paradigms, and the relationship between dependence/withdrawal and tolerance needs to be clarified.

Tolerance in these experiments was seen as rightward and downward shifts in dose-effect curves. This phenomenon has been seen in other tolerance experiments (e.g., Blasig *et al.*, 1979), where increasing opioid tolerance first resulted in a parallel shift to the right of the dose-response curve, and as even more tolerance developed, further rightward, and also downward, shifts were seen. This effect can be explained by phenomena such as receptor down-regulation or receptor desensitization (for a review see Cox, 1990). Whether such phenomena in the dopamine system account for the present observation is unknown, and biochemical studies using appropriate chronic dosing regimens are necessary to confirm or deny their role.

There are several implications of the present data. First, these data support the hypothesis that drugs that substitute for cocaine in a discrimination paradigm will produce cross-tolerance to cocaine when administered in high doses for an extended period of time. Second, the cross-tolerance profile of CNS stimulants in the discrimination paradigm is similar to that of the self-administration paradigm, suggesting that tolerance in the drug discrimination paradigm may be a good predictor of tolerance also occurring to the reinforcing effects of drugs. Finally, to the extent that discrimination of cocaine predicts subjective effects of cocaine in humans (Overton, 1987; Preston and Bigelow, 1991), and to the extent that self-administration of cocaine predicts human cocaine taking (Johanson and Fischman, 1989), the results of the present experiment suggest that drug abusers taking high doses of amphetamines may show cross-tolerance to the subjective and reinforcing effects of cocaine.

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CHAPTER 6

The experiments in this chapter were designed to test specific aim #8: To determine if the *chronic* administration of DA *antagonists* will result in *cross-sensitization* to the reinforcing effects of cocaine. The hypothesis is that the chronic administration of dopamine agonists will result in a shift of the dose-response curve for cocaine self-administration to the left, indicating cross-sensitization to the reinforcing effects of cocaine. If sensitization to cocaine involves an increase in either the concentration or affinity of dopamine receptors, then chronic treatment with dopamine receptor antagonists should stimulate an increase in dopamine receptor activity that results in sensitization to cocaine's reinforcing effects. A final form of this manuscript will be submitted to *Pharmacol. Biochem. Behav.*

Chronic dopamine antagonists produce either cross-sensitization or cross-tolerance to the reinforcing effects of cocaine[1]

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Abbreviations:

flupenthixol (FLU)

Eticlopride (ETI)

SCH23390 (SCH)

fixed ratio (FR)

injection (inj)

inter-reinforcer time (IS^RT)

ABSTRACT

These experiments tested the hypothesis that the chronic administration of the dopamine (DA) antagonists flupenthixol (FLU), SCH23390 (SCH) and eticlopride (ETI) would produce sensitization to the reinforcing effects of cocaine. Rats (N=18) were implanted with indwelling jugular catheters and were trained to self-administer cocaine under a fixed-ratio 2 (FR2) schedule of reinforcement. After stable patterns of self-administration were observed, baseline dose-response curves were obtained. Rats were then treated chronically with either the nonspecific dopamine antagonist FLU (3.2 mg/kg/12 hr/5 days; SC), the specific D1 antagonist SCH (0.25 mg/kg/12 hr/7 days; SC), or the specific D2 antagonist ETI (0.25 mg/kg/12 hr/7 days; SC). Either twenty-four (SCH and ETI) or seventy-two (FLU) hours following the last chronic injection, dose-response curves for cocaine self-administration were obtained. Chronic administration of FLU and SCH shifted the dose-response curve for cocaine self-administration to the left, indicating cross-sensitization to the reinforcing effects of cocaine. In contrast, chronic treatment with ETI shifted the dose-response curve for cocaine self-administration to the right, indicating cross-tolerance to the reinforcing effects of cocaine. In summary, chronic treatment with the mixed dopamine antagonist FLU and the D1 antagonist SCH produce cross-sensitization to the reinforcing effects of cocaine, while chronic treatment the D2 antagonist ETI produced cross-tolerance; indicating that both the D1 and the D2 receptor subtypes

are involved in mediating the reinforcing effects of cocaine; however, they may not contribute to these effects equally.

INTRODUCTION

Cocaine inhibits the reuptake of dopamine, serotonin and norepinephrine (Taylor and Ho, 1978). It has been demonstrated with self-administration experiments that dopamine plays an important role in mediating the reinforcing effects of cocaine. For example, a decrease in the rate of cocaine self-administration is produced by the destruction of central dopamine-containing neurons with the neurotoxin 6-hydroxydopamine (Roberts *et al.*, 1977, 1980; Roberts and Koob, 1982; Pettit *et al.*, 1984). In addition, increased rates of cocaine self-administration are produced by pretreatment with various dopamine antagonists including, the mixed D1/D2 antagonist flupenthixol (Ettinberg *et al.*, 1982; Roberts and Vickers, 1984), the D1 antagonist SCH23390 (Britton *et al.*, 1991; Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993) and the D2 antagonist eticlopride (Caine and Koob, 1994).

An increase in response rate for cocaine self-administration has been interpreted as a reduction in the reinforcing properties of cocaine, because similar

increases in response rate are also observed following reductions in the unit dose of cocaine (Caine and Koob, 1994; de Wit and Wise, 1977; Emmett-Oglesby *et al.*, 1993, Peltier *et al.*, 1996; Pickens and Thompson, 1968).

Although there is a substantial database describing the effects of dopamine antagonists on cocaine self-administration, the majority of these reports examine the acute effects of these compounds. In fact, only a limited number of reports describe chronic treatment effects of dopamine antagonists (Richardson *et al.*, 1994; Roberts and Vickers, 1987). Chronic haloperidol injections (0.075 mg/kg/day for 1 week; IP) produce a progressive increase in cocaine self-administration (Roberts and Vickers, 1987). Similarly, one IM injection of the deconate form of flupenthixol (2.0 mg) or haloperidol (2.5 mg) produces a progressive increase in cocaine self-administration in a low value fixed-ratio paradigm (Richardson *et al.*, 1994). Both haloperidol and flupenthixol are mixed D1/D2 antagonists; therefore, these reports do not demonstrate the contribution of the individual receptor subtypes. The aim of the present experiment is to determine the effects of chronically administered dopamine antagonists on cocaine self-administration, starting with the nonspecific dopamine antagonist flupenthixol (FLU). In addition we will determine the individual involvement of: 1) the D1 receptor subtype by examining the effects of chronic treatment with SCH23390 (SCH); 2) and the D2 receptor subtype by examining the effects of chronic treatment with eticlopride (ETI).

METHODS

Subjects. Subjects, male Fisher F-344 rats, were housed singly and maintained at 270 g ± 10 g by restricting their access to food. For all subjects, water was available *ad libitum* outside of training and testing periods. Subjects were housed in a temperature-controlled room under a 12-hour on/off light cycle.

Training. For a detailed description of the apparatus as well as the training and testing procedures see Emmett-Oglesby *et al.* (1993). Briefly, rats were implanted with an indwelling catheter inserted into the right external jugular, the free end of which was fixed to the skull. After recovery, subjects were given the opportunity to self-administer cocaine on an FR1 schedule with a maximum of 25 cocaine infusions. Once rats self-administered all 25 infusions within 3 hr during two consecutive training sessions, they were then switched to an FR2 training schedule. Before each self-administration session, patency of the catheter was assessed by drawing blood into the catheter and then by flushing 0.1 ml of heparinized saline back into the catheter.

The FR2 training schedule had a maximum of 15 reinforcers and was limited to 3 hr. Rats were tested once a stability criterion was met. This criterion was defined as the average time occurring between reinforcers (the inter-

reinforcer time; $IS^{R}T$, in min.) not varying by more than 20% across three consecutive training sessions.

Testing. Rats (n=18) were tested using a multi-dose procedure. With this procedure, three doses of cocaine (0.125, 0.25 and 0.5 mg/infusion) were available for self-administration during a single test session. This test session consisted of a standard priming infusion (0.3 mg) followed by 24 infusions. These 24 infusions were divided into three blocks of 8 infusions each, with the first block of eight reinforcers containing 0.5 mg/infusion of cocaine, the second containing 0.25 mg/infusion and the third containing 0.125 mg/infusion. Cocaine self-administration tests were conducted in a descending order of cocaine doses (0.5, 0.25, 0.125 mg/kg/infusion); testing was initiated with the high dose in order to increase the probability that the rats begin self-administration. Baseline dose-response curves for cocaine self-administration were obtained 24-hr before the chronic treatment. Twenty-four hours after the last chronic injection of SCH or ETI, post-chronic dose-response curves for cocaine self-administration were obtained. FLU has a very long half-life (16 hours); therefore, post-chronic dose-response curves for rats receiving FLU were obtained 72 hours after the last chronic injection.

Chronic Treatment Regimen. After baseline dose-response data were obtained, all training and testing were suspended. During this time, all rats received SC injections of either FLU (3.2 mg/kg/12 hr/5 days), SCH (0.25 mg/kg/12

hr/7 days) or ETI (0.25 mg/kg/12 hr/7 days). Twenty-four hours after the last chronic injection of SCH or ETI, post-chronic dose-response curves for cocaine self-administration were obtained. FLU has a very long half-life (16 hours); therefore, post-chronic dose-response curves for rats receiving FLU were obtained 72 hours after the last chronic injection.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC) was dissolved in heparinized saline (0.5 U/ml) and filtered through 0.22 µm filters (Millipore, Bedford, MA) into sterile 10 ml syringes immediately before use. FLU, SCH, and ETI (Sigma, St. Louis, MO) were dissolved in water and injected SC.

Data Analysis. In FR2 self-administration experiments, data were scored as the average time between the administration of consecutive injections of cocaine (inter-reinforcer interval, $IS^{R}T$) and analyzed using a two-way repeated measures Analysis of Variance (ANOVA) with treatment condition and dose of cocaine as within subject factors. Data were analyzed using the SYSTAT statistical software package (Wilkinson *et al.*, 1992)

RESULTS

Increasing the dose of cocaine resulted in an orderly increase in the time between injections ([$F_{(2,10)}$ = 54.512; $p<0.001$]; [$F_{(2,10)}$ =12.9; $p<0.01$]; [$F_{(2,16)}$ =134.905; $p<0.001$]) (Figures 1, 2 and 3). Chronic treatment with FLU (3.2 mg/kg/12 hr for 5 days, SC; Figure 1) shifted the cocaine self-administration curve significantly to the left [$F_{(1,5)}$ = 21.256; $p< .01$]. Similarly, chronic treatment with SCH (0.25 mg/kg/12 hr for 7 days, SC; Figure 2) shifted the cocaine self-administration curve significantly to the left [$F_{(1,5)}$ = 17.332; $p< 0.01$], and chronic treatment with ETI (0.25 mg/kg/12 hr for 7 days, SC; Figure 3) shifted the co-caine self-administration curve significantly to the right [$F_{(1,8)}$ = 12.343; $p< 0.01$].

FIGURES

Figure 1.

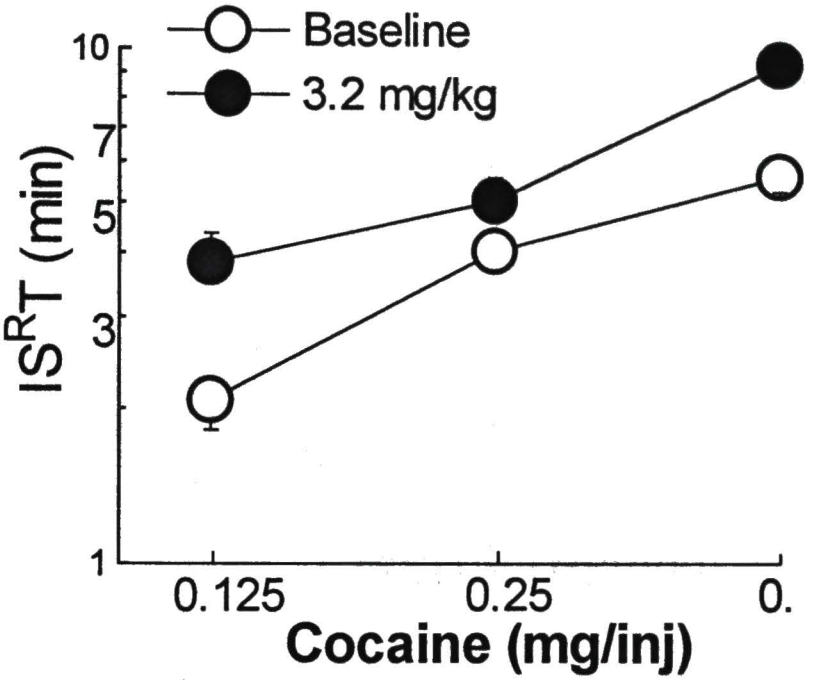

Fig. 1: The effects of chronic treatment with FLU (3.2 mg/kg/12 hr for 5 days; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed circles indicate the dose-response curve for cocaine self-administration 72 hours after the last chronic injection of FLU. Data are represented as group means (n=6) $\pm$ S.E.M.

Figure 2.

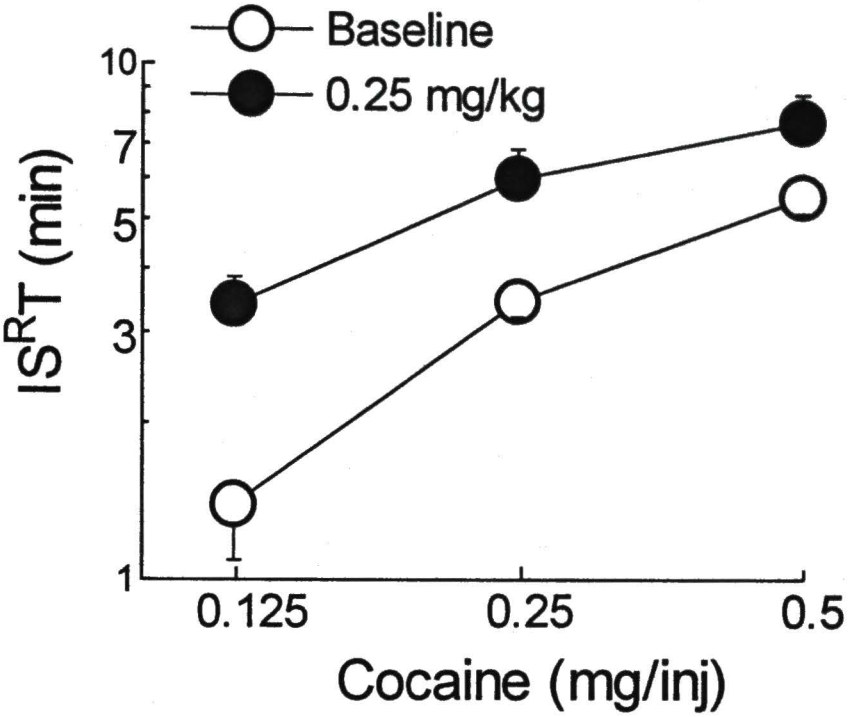

Fig. 2: The effects of chronic treatment with SCH (0.25 mg/kg/12 hr for 7 days; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed circles indicate the dose-response curve for cocaine self-administration 24 hours after the last chronic injection of SCH. Data are represented as group means (n=6) $\pm$ S.E.M.

Figure 3.

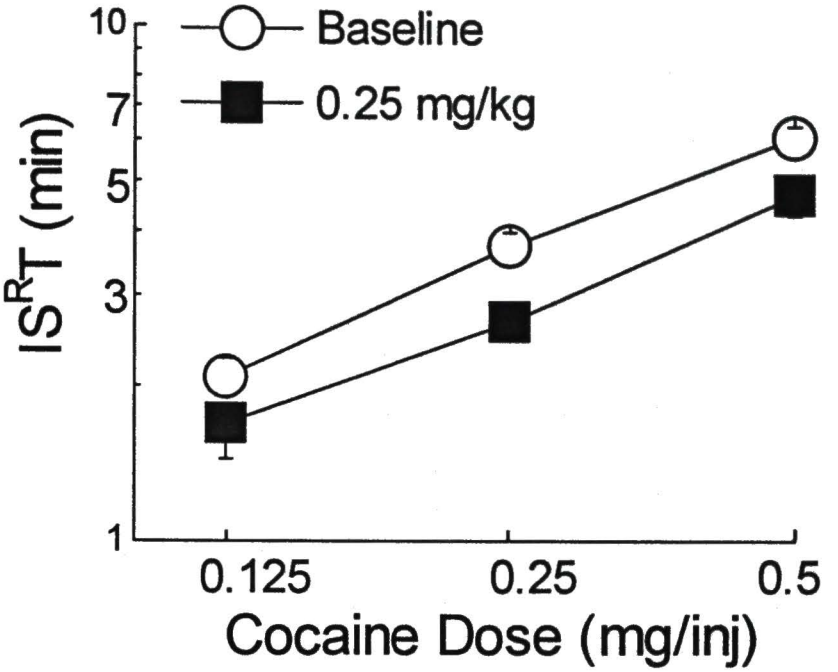

Fig. 3: The effects of chronic treatment with ETI (0.25 mg/kg/12 hr for 7 days; SC) on cocaine self-administration in rats. Abscissa: Dose of cocaine (mg/injection). Ordinate: Time between reinforcers (IS^RT; min.). Open circles indicate the dose-response curve for cocaine self-administration before chronic treatment (baseline). Closed circles indicate the dose-response curve for cocaine self-administration 24 hours after the last chronic injection of ETI. Data are represented as group means (n=6) $\pm$ S.E.M.

DISCUSSION

Chronic treatment with the mixed D1/D2 dopamine antagonists FLU produced cross-sensitization to cocaine under an FR2 schedule of cocaine self-administration. FLU (3.2 mg/kg/12 hr for 5 days) produced between a one- to two-fold shift to the left in the dose-response curve for cocaine self-administration. Previous reports, that treatment with the deconate form of FLU (2.0 mg) produced a progressive increase in the amount of cocaine self-administered (Richardson *et al.*, 1994), differ from the present findings. The differences may be explained by relative drug levels attained in chronic treatment regimens. For example, Richardson and colleagues (1994) administered FLU in a single IM injection that contained 2.5 mg/kg of FLU suspended in oil which produces slow a onset and offset of relatively low peak plasma concentrations. In the present experiment, 3.2 mg/kg of FLU was dissolved in water and injected SC every 12 hours for 5 days, which would result in a larger amount of FLU present all times with the peak plasma concentration being reached more rapidly and maintained at a relatively constant level for an extended period of time. Therefore, it may be that the present results reflect a compensatory neuronal adaptation produced by constant exposure to a high dose of FLU.

Chronic treatment with the D1 dopamine antagonist SCH also produces cross-*sensitization* to cocaine under an FR2 schedule of cocaine self-administration. SCH (0.25 mg/kg/12 hr for 7 days) produced a one- to two-fold

shift to the left in the dose-response curve for cocaine self-administration. In contrast, chronic treatment with the D2 dopamine antagonists ETI (0.25 mg/kg/12 hr for 7 days) produced cross-*tolerance* to cocaine under an FR2 schedule of cocaine self-administration. These results suggest that the D1 and the D2 dopamine receptors are independently involved in the reinforcing effects of cocaine.

One explanation for the present results, is that chronic treatment with dopamine antagonists that act on the D1 receptor up-regulate post-synaptic dopamine D1 receptors, producing effects that are similar to an increase in the unit dose of cocaine. In addition, chronic treatment with dopamine antagonists that act on the D2 receptor up-regulate pre-synaptic dopamine D2 receptors, producing effects that are similar to an decrease in the unit dose of cocaine through feedback inhibition of dopamine release.

This could explain why both FLU and SCH produce different degrees of cross-sensitization to the reinforcing effects of cocaine in the present study. FLU is a mixed D1/D2 dopamine antagonist and SCH is a specific D1 antagonist, therefore, one would expect chronic treatment with SCH to produce a greater degree of cross-sensitization to the reinforcing effects of cocaine. In fact, that is what the present data show. Chronic treatment with SCH resulted in a larger shift of the dose-response curve for cocaine self-administration to the left than did chronic treatment with FLU.

It has been demonstrated that chronic treatment with FLU results in an increase or up-regulation of dopamine D2 receptors (Murugaiah *et al.*, 1983). In that study, FLU (0.8 - 1.2 mg/kg/day) was administered in the drinking water for 18 months. Initially, FLU treatment resulted in a decrease in the B_{max} of D_2 receptors by 40%; however, after 18 months, the B_{max} of D_2 receptors was actually increased by 40%. Similarly, chronic administration of ETI also up-regulates dopamine D2 receptors (LaHoste and Marshall, 1991). LaHoste and colleagues (1991) injected rats daily with ETI (0.5 mg/kg; IP) for 21 days. Ninety-six hours after the last ETI injection, they found that D2 dopamine receptor density was increased by 46% in the caudate-putamen and by 39% in the nucleus accumbens. The difference in the results between ETI and FLU, is that FLU also blocks D1 receptors. It is possible that the blockade of both D1 and D2 receptors can produce opposing effects, so that, if SCH shifts the dose-response curve for cocaine self-administration to the left and ETI shifts the dose-response curve for cocaine self-administration to the right, then the dose-response curve for cocaine self-administration obtained after chronic treatment with FLU should lie somewhere between the two other curves. Where the FLU curve is, in relation to the SCH and ETI curves, should provide us with information concerning the amount of involvement contributed by each receptor subtype in determining the reinforcing efficacy of cocaine.

In summary, chronic treatment with dopamine antagonists that act on the D1 receptor subtype produce cross-sensitization to the reinforcing effects of cocaine in rats, probably by producing an up-regulation of post-synaptic dopamine receptors. In contrast, chronic treatment with dopamine antagonists that act on the D2 receptor subtype produce cross-tolerance to the reinforcing effects of cocaine in rats, probably by producing an up-regulation of pre-synaptic dopamine receptors.

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DISCUSSION

To summarize the data contained in these manuscripts, we have developed a multi-dose method for the determination of a dose-response curve for cocaine self-administration during a single session. The dose-response curve for cocaine self-administration obtained using this method are almost superimposable to those obtained using the more traditional single-dose method. The multi-dose method provides dose-response data that are reproducible. In addition, this method was shown to be an effective method for the detection of tolerance to the reinforcing effects of cocaine.

Chronic cocaine (20 mg/kg/8 hr/for 7 days) administered by the IP route produced a similar degree of tolerance to the reinforcing effects of cocaine as is produced by chronic cocaine delivered by the IV route. In addition, chronic cocaine (20 mg/kg/8 hr/for 7 days; IP) did not increase the rate of cocaine metabolism as measured by plasma and brain levels of cocaine and benzoylecgonine, indicating that this type of pharmacokinetic tolerance is not responsible for producing the behavioral effects observed after chronic cocaine treatment.

With the exception of one dose of *d*-amphetamine, acute pretreatment with dopamine agonists did not affect the rate of cocaine self-administration. In

contrast, acute pretreatment with dopamine antagonists blocked the reinforcing effects of cocaine.

Chronic treatment with the indirect dopamine agonists *d*-amphetamine and methamphetamine produced cross-tolerance to the reinforcing effects of cocaine; while chronic treatment with the direct dopamine agonist apomorphine failed to alter the reinforcing effects of cocaine.

Chronic treatment with the mixed dopamine antagonist flupentixol and the D1 antagonist SCH 23390 produced cross-sensitization to the reinforcing effects of cocaine, while chronic treatment with the D2 antagonists eticlopride produced cross-tolerance to the reinforcing effects of cocaine.

Single-Dose / Multi-Day vs. Multi-Dose / Single-Day Test Procedures

In Chapter 1, it was demonstrated that a 10-day regimen of cocaine, 20 mg/kg per 8 h, produced nearly maximal tolerance within 48 h (Fig. 1). However, the maximal effect of tolerance began decreasing by the second day after chronic cocaine was terminated. In this laboratory's previous report of tolerance to the effects of cocaine (Emmett-Oglesby and Lane, 1992), subjects received 20 mg/kg per 8 h of cocaine for a week, and tolerance was found to extend over

4 days following termination of chronic cocaine. The time course of tolerance loss in the present experiment is consistent with this previous result.

One of the aims of these experiments was to develop a method that would permit more rapid collection of dose-response data in a single session. This would allow us to measure the maximal amount of produced by chronic cocaine treatment (this occurs 24 hr after the last chronic cocaine injection; Chapter 1, Fig. 1). To do this, we modified the method of Winger *et al.* (1989) for use in rats. The multi-dose method in these experiments was accomplished by using different syringes for each dose of cocaine, and switching from one syringe to another to change doses delivered. This method produces a constant volume and flow rate for all injections, which can be contrasted to the method of Winger *et al.* (1989), who delivered different doses of cocaine by using a single syringe and varying the volume of drug solution that was injected. In the present study, we were concerned that such a procedure might produce very low (or very high) injection volumes, which could modify cocaine absorption. Consequently, we elected to use multiple pumps and maintain all injections at a fixed volume of 0.1 ml.

We first compared data obtained from the multi-dose method with data obtained from our single-dose method. The two methods produced essentially inter-changeable dose-response curves, and to our knowledge this is the first demonstration that single-dose and multi-dose testing methods produce compa-

rable data. In addition, the multi-dose method appears to be reliable with regard to replication of data across tests, since three repetitions of the cocaine dose-response curve produced essentially interchangeable results. Thus, this method yields reproducible data, and it does so in a much shorter time than is required by single-dose methods.

Finally, the effect of chronic cocaine, 20 mg/kg per 8 h for 7 days produced no significant change from the baseline dose-response data. These data extend findings reported by Emmett-Oglesby and Lane (1992), who used single-dose testing methodology and found approximately the same magnitude of tolerance to self-administered cocaine following this same dosing regimen of chronic cocaine. Thus, the multi-dose method appears to be particularly useful in studies of tolerance.

Pharmacokinetic vs. Pharmacodynamic Tolerance

In Chapter 1, it was demonstrated that chronic cocaine (20 mg/kg/8 hr/for 7 days) produces tolerance to the reinforcing effects of cocaine (Fig. 3). There are three mechanisms that could account for these findings: tolerance could occur to the behavior-disrupting effects of cocaine, tolerance could occur via pharmacokinetic mechanisms, or tolerance could occur to the reinforcing efficacy of cocaine.

The first possibility, tolerance to the behavioral-disrupting effects of cocaine, stems from the hypothesis that rate of drug self-administration is limited by behavior-disrupting effects of the reinforcer. According to this hypothesis, subjects would take more drug per unit time simply because they are physically capable of taking more drug. This hypothesis leads to an interesting prediction concerning the lowest dose of cocaine that subjects self-administer. If indeed the shorter IS^RTs observed following chronic treatment with cocaine were due to tolerance to the behavior-disrupting effects, then one would expect to see that a low dose of cocaine, which barely maintains cocaine self-administration under baseline conditions, would result in greater cocaine intake following chronic treatment with cocaine. Instead, the entire dose-effect curve was shifted nearly twofold to the right by chronic cocaine, and the lowest dose that barely maintained self-administration before chronic cocaine was not self-administered after chronic cocaine (Emmett-Oglesby and Lane, 1992). In addition, the role of behavior-disruptive effects in the control of drug self-administration is minimized by the observation that are capable of emitting operant responses for alternative reinforcers during the time between self-administration injections of psychostimulants (Wise *et al.*, 1977). The third hypothesis, that tolerance occurs to the reinforcing effect of cocaine, is consistent with both our previous results (Emmett-Oglesby and Lane, 1992) as well as data from the present study.

The second possibility, pharmacokinetic factors, stems from the hypothesis that rate of drug self-administration is decreased as the metabolism rate of cocaine is increased. This could occur because an increase in the rate of cocaine metabolism could result in a decrease in the plasma concentration of cocaine; therefore, there would be a lower concentration of cocaine in the brain.

In Chapter 2, we demonstrated that chronic IP treatment with cocaine (20 mg/kg/8 hours for 7 days) produced a two-fold shift to the right of the dose-response curve for cocaine self-administration, indicating tolerance to the reinforcing effects of cocaine. These results are in concordance with those obtained by previously in this laboratory (Emmett-Oglesby *et al.*, 1993). In the previous study, the chronic cocaine treatment regimen consisted of IV injections, while in the present study, the chronic cocaine treatment regimen consisted of IP injections. All other procedures for the two experiments were identical, and both routes of administration produced similar degrees of tolerance to the reinforcing effects of cocaine.

In Chapter 2, we demonstrated that chronic cocaine treatment failed to increase the plasma concentration of benzoylecgonine at any time point tested, indicating that chronic cocaine did not produce an increase in the metabolism of cocaine (Fig. 1). In the group of rats that was treated chronically with cocaine, there was; however, an increase in the amount of cocaine present in the blood, the first time that we examined the concentration of cocaine in the blood, 15 min

after the IV injection of 2.0 mg/kg of cocaine. In addition, chronic cocaine treatment failed to alter the concentration of cocaine or benzoylecgonine in the brain found after an acute challenge with 2.0 mg/kg of cocaine, IV.

These results are consistent with previous studies that reported either no differences or small differences in plasma and brain levels of cocaine in chronically treated subjects (Ferko *et al.*, 1990; Katz *et al.*, 1993; Nayak *et al.*, 1976).

Acute Treatment with DA agonists and antagonists

In Chapter 3, we demonstrated that acute pretreatment with the *direct* dopamine agonist apomorphine failed to alter the rate of cocaine self-administration (Fig. 1). Similarly, with the exception of one dose of methamphetamine (1.8 mg/kg), acute pretreatment with the *indirect* dopamine agonists *d*-amphetamine (Fig. 2) and methamphetamine (Fig. 3) also failed to alter the rate of cocaine self-administration in rats. It is worth mentioning; however, that acute pretreatment with these indirect dopamine agonists produced behavioral intoxication that was manifested as a dose dependent increase in the delay of initiation of the self-administration behavior.

There are a limited number of other reports describing the effects of acute pretreatment with dopamine agonists on cocaine or *d*-amphetamine self-administration (Pickens *et al.*, 1968; Wilson and Schuster, 1973). In those ex-

periment, acute pretreatment with *d*-amphetamine resulted in a decrease in the rate of either cocaine or *d*-amphetamine self-administration. The present results using a pretreatment of methamphetamine (1.8 mg/kg) produced a shift to the left of the dose-response curve for cocaine self-administration; which is the same as decreasing the rate of cocaine self-administration. Therefore, at least one dose of methamphetamine tested (1.8 mg/kg) produced data that was in concordance with other reports in the literature.

In contrast to the effects of acute pretreatment with dopamine agonists, the dopamine antagonists flupentixol, SCH 23390 and eticlopride all produced a dose dependent increase in the rate of cocaine self-administration, indicating the blockade of the reinforcing effects of cocaine. These data are in concordance with other reports in the literature demonstrate a blockade of the reinforcing effects of cocaine by dopamine antagonists (Britton *et al.,* 1991; Caine and Koob, 1994; Emmett-Oglesby *et al.*, 1993; Ettinberg *et al.*, 1982; Roberts and Vickers, 1984). In these reports, dopamine antagonists produce an increase in the rate of cocaine self-administration that is the same as that produced by a reduction of the unit dose of self-administered cocaine. This is similar to the present results, where pretreatment with the dopamine antagonists flupentixol, SCH 23390 and eticlopride decreased the IS^RTs for cocaine self-administration, which is what occurs when the unit dose of cocaine is decreased. In summary, D1 and D2 dopamine antagonists increase the rate of cocaine self-administration, providing

evidence that both of these receptor subtypes are involved in mediating the reinforcing effects of cocaine, but simultaneous blockade of both receptors is not required to blockade of these effects.

Chronic Treatment with DA Agonists

In Chapter 5, we demonstrated that chronic treatment with *d*-amphetamine produced cross-tolerance to cocaine under an FR2 schedule of cocaine self-administration (Fig. 2). In contrast to the lower doses of *d*-amphetamine, the highest dose of *d*-amphetamine tested, 3.2 mg/kg, shifted the dose-response curve for cocaine self-administration approximately two-fold to the right. The degree of cross-tolerance produced by *d*-amphetamine (3.2 mg/kg/8 hr) in this study was similar to the degree of tolerance produced by cocaine (20 mg/kg/8 hr) under a similar experimental protocol (Emmett-Oglesby *et al.*, 1993).

Chronic treatment with methamphetamine also produced cross-tolerance to cocaine under an FR2 schedule of cocaine self-administration. These results were similar to those obtained with chronic *d*-amphetamine treatment in that the two lowest doses of methamphetamine given chronically, 0.32 and 1.0 mg/kg, did not significantly alter the dose-response curve for cocaine self-administration (these doses also resembled chronic saline treatment). However, the highest

dose of methamphetamine tested, 3.2 mg/kg, did shift the dose-response curve for cocaine self-administration approximately two-fold to the right.

Tolerance in these experiments was seen as rightward and downward shifts in dose-effect curves. This phenomenon has been seen in other tolerance experiments (e.g., Blasig *et al.*, 1979), where increasing opioid tolerance first resulted in a parallel shift to the right of the dose-response curve, and as even more tolerance developed, further rightward, and also downward, shifts were seen. This effect can be explained by phenomena such as receptor down-regulation or receptor desensitization (for a review see Cox, 1990). Whether such phenomena in the dopamine system account for the present observation is unknown, and biochemical studies using appropriate chronic dosing regimens are necessary to confirm or deny their role. To the extent that self-administration of cocaine predicts human cocaine taking (Johanson and Fischman, 1989), the results of the present experiment suggest that drug abusers taking high doses of amphetamines may show cross-tolerance to the subjective and reinforcing effects of cocaine.

In Chapter 4, chronic treatment with the direct dopamine agonist apomorphine failed to significantly alter the dose-response curve for cocaine self-administration. These results are in contrast to the results described above (Chapter 5), where chronic treatment with the indirect dopamine agonists *d*-amphetamine and methamphetamine produced cross-tolerance to the reinforcing

effects of cocaine. In addition, the data obtained after chronic treatment with apomorphine are in contrast to data obtained in a similar experimental paradigm, when rats were treated chronically with the indirect dopamine agonist cocaine (Emmett-Oglesby and Lane, 1992; Emmett-Oglesby *et al.*, 1993). In those experiments, cocaine (20 mg/kg/8 hr/ 7 days; IV) treatment produced tolerance to the reinforcing effects of cocaine. The discrepancy in these findings may be due to the difference in the mechanism of action for cocaine and apomorphine. Cocaine is an indirect dopamine agonists that acts by blocking the reuptake of dopamine (Taylor and Ho, 1978), which results in an increase in the amount of dopamine in the synapses. In contrast, apomorphine is a direct dopamine agonist. It may be possible that long term stimulation of post-synaptic dopamine receptors by dopamine itself is required for the development of tolerance to the reinforcing effects of cocaine.

The current results are also in contrast to those obtained by Wood (1987), who found that chronic treatment with apomorphine produced cross-tolerance to the discriminative stimulus effects of cocaine. In that study, apomorphine (2.5 mg/kg/12 hr/7 days; SC) produced a two-fold shift to the right in the dose-response curve for cocaine discrimination. There are several possible reasons that the results obtained in that experiment differ from those obtained in this experiment. The first reason, is that these two paradigms measure qualitatively different effects of cocaine. The drug discrimination paradigm measures

the subjective effects of drugs, while the self-administration paradigm is a direct measure of the reinforcing properties of a drug. It is unlikely that this is the reason for the discrepancy in these results; however, because there has been very good concordance between results obtained in the drug discrimination and the drug self-administration procedures.

The second possible reason, is the different strains used in the two studies. In the drug discrimination study, Long-Evens rats were used, while in the present study, Fisher F-344 rats were used. It has been recently demonstrated that different strains of rats have different patterns of cocaine self-administration as well as differential responsiveness to acute treatment with dopamine antagonists (Ward *et al.*, 1996). The third possible reason is the difference in the dose of chronic apomorphine. In the discrimination study, 2.5 mg/kg apomorphine was injected every 8 hr for 7 days; while in the present study, 0.32, 1.0 or 3.2 mg/kg apomorphine was injected SC every 12 hr for 7 days. It seems likely; however, that with the range of doses tested in the present experiment (0.32, 1.0 and 3.2 mg/kg), that any effects in the self-administration of cocaine produced by chronic apomorphine would have been detected. In summary, in contrast to the effects of chronic treatment with <u>indirect</u> dopamine agonists, chronic treatment with the <u>direct</u> dopamine agonists apomorphine failed to alter the reinforcing effects of cocaine in rats.

Chronic Treatment with DA Antagonists

In Chapter 6, we demonstrated that chronic treatment with the mixed D1/D2 dopamine antagonists flupentixol produced cross-sensitization to cocaine under an FR2 schedule of cocaine self-administration. Flupentixol (3.2 mg/kg/12 hr for 5 days) produced between a one- to two-fold shift to the left in the dose-response curve for cocaine self-administration. Previous reports, have demonstrated that treatment with the deconate form of flupentixol (2.0 mg) produced a progressive increase in the amount of cocaine self-administered (Richardson *et al.*, 1994), differ from the present findings. The differences may be explained by relative drug levels attained in chronic treatment regimens. For example, Richardson and colleagues (1994) administered flupentixol in a single IM injection that contained 2.5 mg/kg of flupentixol suspended in oil which produces slow a onset and offset of relatively low peak plasma concentrations. In the present experiment, 3.2 mg/kg of flupentixol was dissolved in water and injected SC every 12 hours for 5 days, which would result in a larger amount of flupentixol present all times with the peak plasma concentration being reached more rapidly and maintained at a relatively constant level for an extended period of time. Therefore, it may be that the present results reflect a compensatory neuronal adaptation produced by constant exposure to a high dose of flupentixol.

Chronic treatment with the D1 dopamine antagonist SCH 23390 also produces cross-*sensitization* to cocaine under an FR2 schedule of cocaine self-administration. SCH 23390 (0.25 mg/kg/12 hr for 7 days) produced a one- to two-fold shift to the left in the dose-response curve for cocaine self-administration. In contrast, chronic treatment with the D2 dopamine antagonists eticlopride (0.25 mg/kg/12 hr for 7 days) produced cross-*tolerance* to cocaine under an FR2 schedule of cocaine self-administration. These results suggest that the D1 and the D2 dopamine receptors are independently involved in the reinforcing effects of cocaine.

One explanation for the present results, is that chronic treatment with dopamine antagonists that act on the D1 receptor up-regulate post-synaptic dopamine D1 receptors, producing effects that are similar to an increase in the unit dose of cocaine. In addition, chronic treatment with dopamine antagonists that act on the D2 receptor up-regulate pre-synaptic dopamine D2 receptors, producing effects that are similar to an decrease in the unit dose of cocaine through feedback inhibition of dopamine release.

This could explain why both flupentixol and SCH 23390 produce different degrees of cross-sensitization to the reinforcing effects of cocaine in the present study. flupentixol is a mixed D1/D2 dopamine antagonist and SCH 23390 is a specific D1 antagonist, therefore, one would expect chronic treatment with SCH 23390 to produce a greater degree of cross-sensitization to the reinforcing ef-

fects of cocaine. In fact, that is what the present data show. Chronic treatment with SCH 23390 resulted in a larger shift of the dose-response curve for cocaine self-administration to the left than did chronic treatment with flupentixol.

It has been demonstrated that chronic treatment with flupentixol results in an increase or up-regulation of dopamine D2 receptors (Murugaiah *et al.*, 1983). In that study, flupentixol (0.8 - 1.2 mg/kg/day) was administered in the drinking water for 18 months. Initially, flupentixol treatment resulted in a decrease in the B_{max} of D_2 receptors by 40%; however, after 18 months, the B_{max} of D_2 receptors was actually increased by 40%. Similarly, chronic administration of eticlopride also up-regulates dopamine D2 receptors (LaHoste and Marshall, 1991). La-Hoste and colleagues (1991) injected rats daily with eticlopride (0.5 mg/kg; IP) for 21 days. Ninety-six hours after the last eticlopride injection, they found that D2 dopamine receptor density was increased by 46% in the caudate-putamen and by 39% in the nucleus accumbens.

The difference in the results between eticlopride and flupentixol, is that flupentixol also blocks D1 receptors. It is possible that the blockade of both D1 and D2 receptors can produce opposing effects, so that, if SCH 23390 shifts the dose-response curve for cocaine self-administration to the left and eticlopride shifts the dose-response curve for cocaine self-administration to the right, then the dose-response curve for cocaine self-administration obtained after chronic treatment with flupentixol should lie somewhere between the two other curves.

Where the flupentixol curve is, in relation to the SCH 23390 and eticlopride curves, should provide us with information concerning the amount of involvement contributed by each receptor subtype in determining the reinforcing efficacy of cocaine.

To summarize the data in Chapter 6, chronic treatment with dopamine antagonists that act on the D1 receptor subtype produce cross-*sensitization* to the reinforcing effects of cocaine in rats, probably by producing an up-regulation of post-synaptic dopamine receptors. In contrast, chronic treatment with dopamine antagonists that act on the D2 receptor subtype produce cross-*tolerance* to the reinforcing effects of cocaine in rats, probably by producing an up-regulation of pre-synaptic dopamine receptors. By default, the effects of mixed dopamine agonists on the reinforcing effects of cocaine will be dependent on the specificity of that particular compound for either the D1 or the D2 receptor subtype.

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