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**THE EFFECTS OF MDL 72222, KETANSERIN AND METHYSERGIDE
PRETREATMENTS ON COCAINE SELF-ADMINISTRATION IN RATS**

by

Susan Lacosta, B.A., B.Sc.

**A thesis submitted to the faculty of Graduate Studies
in partial fulfilment of the requirements for the degree of
Master of Science**

**Department of Psychology
Carleton University
Ottawa, Ontario
May, 1992**

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Thesis Supervisor

Chairman, Department of Psychology

Carleton University

May, 1992

ABSTRACT

The effects of three serotonin receptor antagonists on cocaine self-administration behaviour were investigated. Specifically, the effects of MDL 72222 (a specific 5-HT₁ receptor antagonist), ketanserin (a specific 5-HT₂ receptor antagonist) and methysergide (an aselective 5-HT₁/5-HT₂ receptor antagonist) on the breaking points reached by rats on a progressive ratio schedule for cocaine reinforcement were studied. Pretreatments with MDL 72222 (7.5 - 1920 ug/kg, SC), ketanserin (0.4 - 6.4 mg/kg, IP) and methysergide (2.5 - 20 mg/kg, IP) failed to alter breaking points from baseline values. These data suggest that relatively specific blockade of 5-HT receptor subtypes does not influence the reinforcing effects of cocaine.

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INTRODUCTION

Cocaine hydrochloride is a potent psychomotor stimulant drug. It is a benzoic acid derived from the leaves of the *Erythroxylum coca* bush of Peru and Bolivia. On the streets, cocaine hydrochloride is often referred to as coke, flake, snow and stardust (Cox et al., 1987).

Cocaine exhibits a variety of pharmacological effects on both the central and sympathetic nervous systems. Centrally, it acts as a potent neural stimulant producing intense feelings of well-being and euphoria, heightening alertness and decreasing the need for sleep (Cox et al., 1987). Activation of the sympathetic nervous system results in increased heart rate, blood pressure and in the constriction of peripheral blood vessels. Along with its stimulant properties, cocaine is a potent local anesthetic, blocking the neural conduction of nerve fibers (Cox et al., 1987).

Cocaine possesses powerful reinforcing properties which have contributed to its widespread abuse. In fact, it appears to be one of the most potent reinforcing drugs known to exist (Griffiths et al., 1975, 1978; Aigner and Balster, 1978; Collins et al., 1984). Its abuse is one of the major drug problems facing society today. In 1986, The National Institute on Drug Abuse estimated that 3 million people regularly abuse cocaine. It has also been estimated that

more than 20 million people in the United States have used cocaine at some time and this number appears to be rising (Goodman and Gillman, 1990). Cocaine is used by individuals from all socioeconomic groups and occupations and its abuse is associated with many disturbing socioeconomic, neuropsychiatric and medical consequences. In order to effectively treat cocaine addiction, the neural mechanisms responsible for cocaine's addictive properties must be clearly understood.

The Effect of Cocaine on Monoamine Systems

Cocaine acts as an indirect agonist at dopamine (DA), serotonin (5-HT) and norepinephrine (NE) neuronal synapses in the brain. Cocaine binds to the transporter of these monoamines and blocks their re-uptake into the presynaptic terminal. The resulting increase in synaptic monoamine concentrations prolongs the duration of receptor stimulation. In this way, cocaine increases the synaptic efficacy of the monoamines without directly stimulating the receptors (McKim, 1986).

Each of the monoamine transmitters is located in particular cell clusters in the pons, medulla and mesencephalon regions of the brain. From here, neuronal projections are sent throughout the central nervous system (CNS) so that most brain regions are innervated by one or

more of the transmitters (Cooper et al., 1986). Since cocaine acts at all of these monoamine transmitter sites, it exerts pharmacological effects throughout the CNS. However, cocaine's effects in only some of these areas are thought to be essential for its reinforcing properties. It remains to be determined how each of the monoamine systems and specific brain regions contribute to the addictive properties of cocaine.

Researchers have focused on the role of monoamines in psychomotor stimulant reinforcement. Much evidence linking cocaine's reinforcing properties to monoamine systems comes from experiments employing the self-administration paradigm. A review of the self-administration literature reveals that DA plays an essential role in the reinforcing properties of cocaine and amphetamine while a similar role for NE has not been found (de Wit and Wise, 1977; Woolverton, 1987). More recently, studies have shown that 5-HT also appears to be important in stimulant self-administration behaviour.

The Role of Dopamine in Stimulant Reinforcement

The importance of DA in the reinforcing effects of psychomotor stimulant drugs has been well established. Specifically, pharmacological studies have investigated the effects of DA agonists and antagonists on the rate of stimulant self-administration. First, pretreatment with the

DA agonists methamphetamine (Pickens et al., 1968), phenmetrazine (Wilson and Schuster, 1973), apomorphine, piribedil (Yokel and Wise, 1978) and bromocriptine (Hubner and Koob, 1990) decreased the rate of stimulant self-administration. Conversely, increases in the rate of stimulant self-administration were found following pretreatment with low doses of the DA antagonists chlorpromazine (Wilson and Schuster, 1972, Risner and Jones, 1976), pimozide (Yokel and Wise, 1975; Risner and Jones 1976; de Wit and Wise 1977), perphenazine (Johanson et al., 1976), butaclamol (Yokel and Wise, 1976), alpha-flupenthixol, sulpiride, metoclopramide, thioridazine (Roberts and Vickers, 1984), spiperone and SCH 23390 (Koob et al., 1987).

The importance of the dopaminergic system in psychomotor stimulant reinforcement is supported by various lesion studies. In these studies, the DA neurotoxin 6-hydroxydopamine (6-OHDA) was injected into various terminal regions and fiber bundles to identify the specific dopaminergic brain areas important in cocaine reinforcement.

The nucleus accumbens septi (NAS) was shown to play a significant role in cocaine reinforcement. Specifically, Roberts et al. (1977) revealed that depletion of DA from the NAS after intra-accumbens infusions of 6-OHDA abolished cocaine self-administration on an FR1 schedule of reinforcement. Koob and his colleagues later confirmed these

findings using a PR schedule of reinforcement (Koob et al., 1987). Similar reductions in cocaine self-administration were demonstrated following kainic acid lesions of the nucleus accumbens (Zito et al., 1985). Cocaine self-administration was also disrupted following 6-OHDA lesions of the ventral tegmental area (VTA), an area which innervates the nucleus accumbens (Robert and Koob, 1982).

Additional support for the role of DA in cocaine reinforcement comes from the receptor binding studies of Ritz et al. (1987). These researchers showed that the binding of cocaine analogues to the DA transporter was both significantly and positively related to their reinforcing potencies as determined from self-administration studies. These findings suggest that the DA transport mechanism may be a major site of action in mediating cocaine's reinforcing properties (Ritz et al., 1987).

In summary, the above pharmacological, lesion and receptor binding studies demonstrate a critical role for DA in psychomotor stimulant reinforcement.

The Role of Norepinephrine in Stimulant Reinforcement

It has generally been accepted that NE does not play a significant role in psychomotor stimulant reinforcement. For example, pretreatment with either the noradrenergic agonist methoxamine (Risner and Jones, 1976) or the noradrenergic

antagonists propranolol (de Wit and Wise, 1977), phentolamine (Wilson and Schuster, 1974; de Wit and Wise, 1977) and phenoxybenzamine (Wilson and Schuster, 1974; Risner and Jones, 1976; de Wit and Wise, 1977), failed to alter the rate of stimulant self-administration. Cocaine self-administration was also not affected by either nisoxetine, a selective NE re-uptake blocker, or prazosin, a selective alpha1 NE receptor blocker (Woolverton, 1987).

Similarly, stimulant self-administration remained unaltered following lesioning of the noradrenergic pathway. Roberts et al. (1977) injected the neurotoxin 6-OHDA into the NE tract, a group of fibers extending from the locus coeruleus to the forebrain via the dorsal midbrain. No significant difference in the rate of cocaine self-administration from prelesion values was found despite the almost complete loss of forebrain NE (Roberts et al., 1977).

The receptor binding studies of Ritz and her colleagues also suggest that NE does not play a critical role in psychomotor stimulant reinforcement. Specifically, no significant relationship was found between the binding potencies of cocaine or amphetamine analogues to the NE transporter and their reinforcing efficacies as determined from self-administration studies (Ritz et al., 1987; Ritz and Kuhar, 1989).

In summary, the above findings demonstrate that while DA plays a critical role in the reinforcing properties of

psychomotor stimulants, NE appears to be of no significance.

The Role of Serotonin in Stimulant Reinforcement

Researchers have recently focused on the role of 5-HT in psychomotor stimulant reinforcement. Lyness and co-workers were the first to demonstrate an important role for 5-HT in amphetamine reinforcement. In their initial study, Lyness et al. (1980) reported that rats depleted of forebrain 5-HT following intraventricular injections of the neurotoxin, 5,7-dihydroxy-tryptamine (5,7-DHT), self-administered more amphetamine than control animals. In a subsequent experiment, Leccese and Lyness (1984) revealed that 5,7-DHT lesions of the median forebrain bundle (MFB) also produced substantial increases in amphetamine intake.

The above lesioning studies have shown that depletion of forebrain 5-HT increases the rate of amphetamine self-administration. As a result, 5-HT appears to play an important role in amphetamine reinforcement.

Pharmacological studies have also investigated the importance of 5-HT in amphetamine self-administration. Specifically, reductions in amphetamine intake have been reported following stimulation of 5-HT receptor activity. Pretreatment with L-tryptophan (a 5-HT precursor) administered either systemically (Leccese and Lyness, 1984) or as a dietary supplement (Smith et al., 1986), decreased

the rate of amphetamine self-administration. Similarly, amphetamine intake was attenuated following pretreatment with either fluoxetine, a specific 5-HT re-uptake blocker (Yu et al., 1983; Leccese and Lyness, 1984; Porrino et al., 1988) or quipazine, a direct 5-HT receptor agonist (Leccese and Lyness, 1984).

Since stimulation of 5-HT receptors appears to diminish amphetamine self-administration, pretreatment with 5-HT receptor antagonists might be predicted to increase amphetamine self-administration behaviour. Unfortunately, the data do not support this idea. Instead, pretreatment with either methysergide (an aselective 5-HT₁/5-HT₂ receptor antagonist), cyproheptadine (a 5-HT₂ receptor antagonist) or cinancerin (a specific 5-HT₂ receptor antagonist) reduced amphetamine intake (Leccese and Lyness, 1984; Porrino et al., 1988).

Receptor binding studies have added to our understanding of 5-HT's role in amphetamine reinforcement. Ritz and Kumar (1989) investigated the ability of amphetamine analogues to compete for binding sites with agents specific for the 5-HT transporter. These authors revealed a negative relationship between the reinforcing efficacy of amphetamine-like compounds (as determined from self-administration studies) and their affinities at 5-HT re-uptake sites. This finding suggests that 5-HT not only

plays an important but perhaps also a negative role in amphetamine reinforcement.

The Role of Serotonin in Cocaine Reinforcement

The above mentioned studies indicate that 5-HT plays a significant role in amphetamine reinforcement. Fewer studies have examined the effects of serotonergic manipulations on cocaine self-administration behaviour although these data appear to be consistent with the amphetamine work.

Specifically, both repeated (Carroll et al., 1990a) and acute (Peltier and Schenk, 1991b) pretreatments with fluoxetine significantly reduced the rate of cocaine intake. Similarly, Carroll et al., (1990b) found that repeated administration of dietary L-tryptophan attenuated the rate of cocaine self-administration.

Since agonist pretreatments decrease the rate of cocaine intake, one might predict an increase in the rate of cocaine self-administration following pretreatment with 5-HT antagonists. Unfortunately, the data do not support this hypothesis. Instead, pretreatment with cinanserin, ritanserin (both 5-HT₂ receptor antagonists) or GR23032F (a 5-HT₂ receptor antagonist) failed to alter the rate of cocaine self-administration (Porrino et al., 1989; Peltier and Schenk, 1991a,b).

In summary, the above studies have demonstrated that

5-HT plays an important role in the reinforcing properties of both amphetamine and cocaine. Unfortunately, the exact nature of its effect on stimulant reinforcement remains inconclusive since difficulties exist in using rate as the dependent measure.

Limitations of Rate as a Measure of Reinforcement

The majority of studies examining psychomotor stimulant self-administration have used rate as the dependent variable. Recently, however, rate of drug self-administration has been criticized for being both an uninterpretable and insensitive measure of drug reinforcement (Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990). For example, increases in response rate have been interpreted as reflecting both an increase (Wilson and Schuster, 1973) and a decrease (Yokel and Wise, 1975) in the reinforcing efficacy of a drug. Since the same data can be taken as evidence in support of either of the two opposing hypotheses, the value of rate as a measure of drug reinforcement is questionable.

Rate is also an insensitive measure of drug reinforcement. For example, changes in the reinforcing efficacy of a drug are not always reflected in changes in the rate of drug self-administration (Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990).

The Progressive Ratio Schedule of Reinforcement

Difficulties in interpreting changes in rate of self-administration have prompted several researchers to employ a progressive ratio (PR) schedule to examine changes in drug reinforcement (Griffiths et al., 1975, 1978, 1979; Bedford et al., 1978; Koob et al., 1987; Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990). In this reinforcement schedule, the response requirements to obtain a drug injection are systematically increased with each successive reinforcement. In addition, the breaking points derived from a PR schedule result in a rate-free measure of drug reinforced responding.

Breaking point, in contrast to rate, is a sensitive measure of drug reinforcement. For example, breaking points were significantly increased following 5,7-DHT lesions of the MFB and amygdala although the rates of cocaine self-administration remained unchanged (Loh and Roberts, 1990). Similarly, 6-OHDA lesions of the nucleus accumbens significantly increased breaking points while the rate of apomorphine self-administration was unaffected (Roberts, 1989). Breaking points are also sensitive to hormonal changes across the estrous cycle while the rate of cocaine intake is not (Roberts et al., 1989a).

The PR schedule was originally suggested by Hodos (1961) as a means of assessing the relative strength of food

reward. Recently, the PR schedule has been shown to be sensitive to changes in dose, pharmacological manipulations and neurotoxic lesions. As a result, it appears to be a viable alternative to the use of the FR schedule in studying the neurochemical basis of stimulant reinforcement (Koob et al., 1987; Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990; Richardson and Roberts, 1991).

To date, the PR schedule has been employed in studies assessing the roles of both DA and 5-HT in cocaine reinforcement. Koob et al., (1987) were the first to examine the effects of dopaminergic manipulations on cocaine self-administration behaviour using a PR schedule of reinforcement. These researchers revealed that 6-OHDA lesions of the nucleus accumbens significantly decreased breaking points. A similar reduction in breaking points was found following both acute (Roberts et al., 1989b) and chronic pretreatments (unpublished data) of haloperidol, a highly specific D2 receptor antagonist. The acute administration of (+)-AJ 76 (a mixed DA agonist/antagonist) also diminished breaking points for cocaine reinforcement (Richardson et al., submitted).

These findings further support the hypothesis that DA plays a critical role in cocaine reinforcement. In addition, the resulting changes in breaking point (as opposed to changes in rate) reveal that DA plays a positive, facilitative role in the reinforcing efficacy of cocaine.

Rational and Objectives

Our lab has recently employed a PR schedule of reinforcement to better assess the role of 5-HT systems in cocaine self-administration (Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990, Richardson and Roberts, 1991; McGregor et al., submitted). For example, Loh and Roberts (1990) found an increase in breaking points following intracerebral infusions of 5,7-DHT into either the MFB or the amygdala. These findings support the idea that the motivation to self-administer cocaine is increased following the removal of 5-HT systems.

Pretreatment with certain pharmacological agents have been found to significantly decrease breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement. Specifically, Richardson and Roberts (1991) showed that pretreatment with fluoxetine (a specific 5-HT re-uptake inhibitor) diminished breaking points for cocaine reinforcement. Similar decreases in breaking points were also found following intravenous administration of L-tryptophan (a 5-HT precursor) (McGregor et al., submitted).

The above studies using a PR schedule of reinforcement have shown that stimulation of 5-HT receptors decreases an animal's motivation to self-administer cocaine. As a result, it might be predicted that under a similar reinforcement

schedule, blockade of 5-HT receptors would diminish the adverse effect of these receptors, thereby increasing the reinforcing efficacy of cocaine. It seems appropriate that investigation of this phenomena should be incorporated into our present understanding of 5-HT's role in cocaine reinforcement.

The present thesis served to examine the roles of three 5-HT receptor antagonists on cocaine self-administration behaviour. Specifically, the effects of MDL 72222 (a specific 5-HT₁ receptor antagonist), ketanserin (a specific 5-HT₂ receptor antagonist) and methysergide (an aselective 5-HT₁/5-HT₂ receptor antagonist) on the breaking points established by rats on a PR schedule for cocaine reinforcement were investigated. Our lab has been contracted by the National Institute on Drug Abuse to evaluate these specific 5-HT receptor antagonists in our animal model of drug abuse.

GENERAL METHODS

Subjects

Male Wistar rats (Charles River Farms, Quebec) weighing approximately 275-300 g at the start of the experiments served as subjects. Rats were individually housed and maintained on a 12 h reversed light-dark cycle (lights off from 9:30 am to 9:30 pm). Animals were given continuous access to both food and water.

Apparatus

Plexiglas boxes (25 X 25 X 25 cm) served as both animal housing and testing chambers. Each box was equipped with a wire grid floor, a removable response lever, a stimulus light placed to the left of the lever and a water bottle. Rats were surgically implanted with chronically indwelling jugular cannulae. The cannulae were attached to tubing encased within a protective spring. The tubing, which was held in suspension above the cage by a counter-balance and swivel, extended to a 10 ml syringe. Infusions of cocaine hydrochloride were delivered to the animals by a Razel syringe driver fitted with a 5 RPM motor. The counter-balanced swivel allowed animals free movement in the testing chamber. Response monitoring, infusion delivery and data recording were controlled by an IBM-compatible computer located in the experimental room.

Procedure

Approximately one week following their arrival from the supplier, rats were food deprived for 24 hours and then trained to press a lever for food reward (Noyes pellets) on a fixed ratio (FR) 1 schedule of reinforcement. In this case, every lever response activated a feeder to deliver a single pellet. Once animals exceeded 100 lever responses in each of two training sessions on the FR1 schedule of reinforcement, they were anaesthetized using sodium pentobarbitol (Somnotol, 60 mg/kg, IP) and surgically implanted with a chronically indwelling jugular cannula exiting from the animal's back at the mid-scapular region (Roberts and Goeders, 1989). Following cannulation, rats were singly housed in operant testing chambers.

Initially, rats were post-operatively trained to respond on a lever for cocaine infusions on an FR1 schedule of reinforcement. Specifically, each lever response resulted in the delivery of a cocaine injection (0.6 mg/0.12 ml saline/injection over 5 seconds). Concurrent with the start of the injection, a stimulus light was activated which signalled a 20 second post infusion time-out period during which time responses produced no programmed consequence. Cocaine self-administration sessions took place for a five hour period daily. Each session began with the introduction of the lever into the testing chamber and a "priming" injection.

After a rat achieved a constant rate of responding on an FR1 schedule of reinforcement (a minimum of three consecutive days in which the total number of daily infusions varied no more than 10%), a PR schedule was imposed. In accordance with this schedule, the first lever response of the session resulted in a drug infusion after which the response requirements necessary to obtain subsequent infusions were incremented following the progression: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. The initial ratio was reset to 1 at the beginning of each session.

Typically, rats will respond during the first three hours of a session but fail to respond during the last two hours when the requirements imposed by the PR schedule become too demanding. At this point in the session, the response requirements exceed the reinforcing efficacy of the drug and the lever pressing behaviour extinguishes. A rat was assumed to have reached its breaking point if it failed to obtain an infusion for a one hour period. In this case, the breaking point was defined as the last increment in the schedule completed prior to a 1 hour period of non-reinforcement. This breaking point serves as the dependent measure.

Once a rat established a consistent baseline level of responding on the PR schedule of reinforcement for three

consecutive days, it was placed into one of three experiments.

Drugs

Cocaine HCl, supplied by NIDA (Bethesda, MD), was prepared in a vehicle solution of 0.9% saline. MDL 72222 (RBI, Natick, Mass) was dissolved in one drop of glacial acetic acid and take to volume with distilled water. The final solution was adjusted to a pH of 5-6 with 1 N NaOH. Ketanserin (RBI, Natick, Mass) was dissolved in 0.9% saline. Methysergide (Sandoz) was dissolved in three to five drops of HCl and taken to volume with distilled water. The final solution was adjusted to a pH of 5-6 with 1 N NaOH. All three 5-HT receptor antagonists were injected in a volume of 1.0 ml/kg of body weight.

Statistical Analyses

All analyses were performed on the breaking points. Mean breaking points established following drug pretreatments were compared with those established the day previous to testing using a 2-way (Treatment X Dose) repeated Analysis of Variance (ANOVA). Treatment served as the within subjects measure and Dose as the between subjects measure.

Experiment 1

The effect of MDL 72222 pretreatment on cocaine self-administration using a PR schedule of reinforcement.

MDL 72222 is a highly selective 5-HT₂ receptor antagonist (Fozard, 1984) which has been used in a variety of drug related experiments. For example, Glaum (1990) showed that MDL 72222 successfully blocks the antinociceptive effects of both 5-HT and 2-Me-5-HT as measured in tail flick and hot plate tests. It has also been found that MDL 72222 dose dependently blocks place aversion elicited by naloxone, PCP and picrotoxin administration (Aguas et al., 1990). Conversely, MDL 72222 antagonizes morphine- (Aguas et al., 1988; Carboni et al., 1989; Higgins et al., 1992), nicotine-, (Aguas et al., 1988; Carboni et al., 1989) and MDMA-induced place preference conditioning (Bilsky and Reid, 1991).

Methods

Subjects

Fourteen male Wistar rats served as subjects in this experiment. Five animals were tested at each dose. Animals were replaced in the event of illness or cannula failure. As a result, not all rats received all doses of drug.

Pretreatment

On the day immediately following three days of consistent baseline on a PR schedule of reinforcement, animals were pretreated with MDL 72222 (7.5, 15, 30, 60, 120, 960 or 1920 ug/kg, SC) 30 minutes prior to the testing session. All drug doses were administered in a counterbalanced fashion. Rats were required to re-establish a stable daily breaking point for three days before being given the next dose of drug.

Experiment 2

The effect of ketanserin pretreatment on cocaine self-administration.

Ketanserin is a highly selective 5-HT₂ receptor antagonist which has been used clinically in the treatments of hypertension and peripheral vascular diseases (Janssen, 1983; Fozard, 1987). Only two papers report using the compound in drug related studies. For example, ketanserin has been shown to significantly block the discrimination of LSD from saline in a two lever drug discrimination task (Cunningham and Appel, 1987). Ketanserin also dose dependently reduces the head shaking behaviour induced by L-5-HTP and the tremors and forepaw treading induced by 5-Me-ODMT (Shearman and Tolcsvai, 1987).

Methods

Subjects

Thirteen male Wistar rats served as subjects in this experiment. Five animals were tested at each dose. Animals were replaced in the event of illness or cannula failure. As a result, not all rats received all doses of drug.

Pretreatment

On the day immediately following three days of consistent baseline on a PR schedule of reinforcement, animals were pretreated with Ketanserin (0.4, 0.8, 1.6, 3.2 or 6.4 mg/kg, IP) 1 hour prior to the testing session. All doses of ketanserin were administered in a counterbalanced fashion. Rats were required to re-establish a stable daily breaking point for three days before being given the next dose of drug.

Experiment 3

The effect of methysergide pretreatment on cocaine self-administration.

Methysergide is a congener of LSD which functions as an aselective 5-HT₁/5-HT₂ receptor antagonist. For the last few decades, it has been used clinically in the treatment of migraines (Fozard, 1987). Methysergide has also been used thoroughly in rats. For example, it has been found to attenuate amphetamine self-administration in rats (Leccese and Lyness, 1984). On the other hand, methysergide potentiates both the rate increasing effects of amphetamine self-stimulation (Silveria Filho and Graeff, 1977) and the hyperactivity produced by MDMA administration (Gold and Koob, 1988).

Methods

Subjects

Thirteen male Wistar rats served as subjects in this experiment. Five animals were tested at each dose. Animals were replaced in the event of illness or cannula failure. As a result, not all rats received all doses of drug.

Pretreatment

On the day immediately following three days of consistent baseline on a PR schedule of reinforcement, animals were pretreated with methysergide (2.5, 5, 10 or 20 mg/kg, IP) 1 hour prior to testing. Animals in this study also received a vehicle injection of distilled water (1 ml/kg). Pretreatments were administered in a counterbalanced fashion. Rats were required to re-establish a stable daily breaking point for three days before being given the next dose of drug.

RESULTS

Figures 1, 2 and 3 show the effects of MDL 72222, ketanserin and methysergide respectively on the breaking points achieved by rats self-administering cocaine on a PR schedule of reinforcement. Analysis using a repeated two-way ANOVA revealed no significant main effects of Treatment or Dose and no significant Treatment by Dose interactions of either MDL 72222: Treatment [$F(1,28) < 1$, NS], Dose [$F(6,28) < 1$, NS], Treatment by Dose [$F(6,28) < 1$, NS] or methysergide: Treatment [$F(1,20) < 1$, NS], Dose [$F(4,20) < 1$, NS], Treatment by Dose [$F(4,20) < 1$, NS]. Similar analysis revealed a significant main effect of Treatment within animals for ketanserin $F(1,20) = 6.05$, $p < 0.05$. This significant Treatment effect is probably not related to ketanserin but to the handling of the animals since no main effect of Dose [$F(4,20) < 1$, NS] or Treatment by Dose interactions [$F(4,20) < 1$, NS] were evident.

Figure 1: The effect of MDL 72222 pretreatment on the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement. The mean ($\pm$ SEM) breaking point values following pretreatment with MDL 72222 (7.5, 15, 30, 60, 120, 960 and 1920 ug/kg, SC) are shown. Five rats were tested at each dose. All values are expressed in terms of percent of baseline performance.

Figure 2: The effect of ketanserin pretreatment on the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement. The mean ($\pm$ SEM) breaking point values following pretreatment with ketanserin (0.4, 0.8, 1.6, 3.2 or 6.4 mg/kg, IP) are shown. Five rats were tested at each dose. All values are expressed in terms of percent of baseline performance.

Figure 3: The effect of methysergide pretreatment on the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement. The mean ($\pm$ SEM) breaking point values following pretreatment with methysergide (2.5, 5, 10 or 20 mg/kg, IP) and vehicle (1 ml/kg) are shown. Five rats were tested at each dose. All values are expressed in terms of percent of baseline performance.

DISCUSSION

Pretreatments with MDL 72222 (7.5 - 1920 ug/kg, SC), ketanserin (0.4 - 6.4 mg/kg, IP) and methysergide (2.5 - 20 mg/kg, IP) failed to alter the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement from baseline values. These data suggest that the relatively specific blockade of 5-HT receptor subtypes does not influence the reinforcing effects of cocaine.

Two separate lines of evidence suggest a role for 5-HT in the reinforcing properties of cocaine. First, 5-HT appears to have a direct action on cocaine reinforcement as apparent from the ability of 5-HT manipulations to alter cocaine self-administration (Lyness et al., 1980; Leccese and Lyness, 1984; Smith et al., 1986; Yu et al., 1986; Porrino et al., 1988; Carroll et al., 1990a,b; Loh and Roberts, 1990; Peltier and Schenk, 1991b; Richardson and Roberts, 1991; McGregor et al., submitted). Second, evidence also suggests that 5-HT may indirectly effect cocaine reinforcement through its ability to directly modulate DA activity (Blandina et al., 1988,1989; Chen et al., 1990; Jiang et al., 1990). These two lines of evidence will now be discussed.

5-HT₂/DA Interactions

5-HT has recently been shown to innervate and modulate the mesolimbic DA system of the brain. Specifically, 5-HT is believed to facilitate the release of DA through the activation of 5-HT₂ receptors (Blandina et al., 1988,1989; Chen et al., 1990; Jiang et al., 1990). Biochemical, pharmacological and behavioral studies support the role of 5-HT₂ receptors in the modulation of mesolimbic DA activity. For example, high levels of 5-HT₂ binding have been identified in the mesolimbic DA pathway from radioligand binding studies (Kilpatrick, 1987).

Pharmacological studies have also shown that 5-HT₂ receptors may modulate the release of DA. For example, DA release from striatal slices following administration of 2-Me-5-HT (a selective 5-HT₂ receptor agonist) was blocked by ICS 205-930 (a 5-HT₂ receptor antagonist) (Blandina et al., 1988,1989). Conversely, 5-HT evoked DA release was unaltered following administration of methiothepin (a 5-HT₂ receptor antagonist) or methysergide (an aselective 5-HT₁/5-HT₂ receptor antagonist). Likewise, neither DOI, a selective 5-HT₂ receptor agonist nor 5-CT, a selective 5-HT₂ receptor agonist effected DA release (Blandina et al., 1989).

Intraventricular administration of 2-Me-5-HT was similarly found to enhance DA release in the nucleus accumbens. This effect was blocked by administration of granisetron (a selective 5-HT₂ receptor antagonist) but not

by metergoline (a 5-HT₁/5-HT₂ receptor antagonist). Furthermore, 8-OHDA (a selective 5-HT_{1A} receptor agonist) and DOI both failed to alter the release of DA (Jiang et al., 1990). Elevated levels of nucleus accumbens DA following perfusion with 1-phenylbiguanide (a selective 5-HT₂ receptor agonist) were also reduced with the selective 5-HT₂ receptor antagonists GR38032F and zacopride (Chen et al., 1991).

Behavioral studies support a modulatory role for 5-HT₂ receptors in DA activity. For example, hyperactivity induced by either infusion of DA into the nucleus accumbens (Costall et al., 1987, 1988) or injection of the neurokinin agonist DiMe-C7 into the VTA (Hagan et al., 1987) was blocked by administration of the selective 5-HT₂ receptor antagonist, GR38032F. Likewise, Reith (1990) showed that cocaine-induced increases in locomotor activity were attenuated by ICS 205-930 and zacopride while methysergide was without effect.

As a result, the above findings suggest that 5-HT₂ receptors modulate the release of mesolimbic DA while 5-HT₁ and 5-HT₂ receptors are without effect. Furthermore, along with the established importance of mesolimbic DA in cocaine reinforcement, 5-HT₂ receptors may be expected to exert their effect on cocaine reinforcement by modulating DA activity. Unfortunately, this hypothesis was not supported by the present study and as such, the modulation of DA by

5-HT₂ receptors does not appear to play a role in the reinforcing properties of cocaine.

5-HT/Cocaine Reinforcement

Aside from the apparent interaction between 5-HT₂ receptors and the mesolimbic DA system, evidence for a direct action of 5-HT on cocaine reinforcement also exists. Several studies employing a PR schedule of reinforcement have demonstrated an important albeit inhibitory role for 5-HT in cocaine reinforcement. For example, increases in breaking points have resulted from bilateral 5,7-DHT lesions of both the MFB and the amygdala (Loh and Roberts, 1990). Thus, the motivation to self-administer cocaine appears to increase following removal of 5-HT systems. These findings are consistent with other 5-HT lesion studies which have reported increases in the rate of amphetamine intake following either intraventricular injections of 5,7-DHT (Lyness et al., 1980) or 5,7-DHT lesions of the MFB (Leccese and Lyness, 1984).

Conversely, the motivation to self-administer cocaine seems to diminish following stimulation of 5-HT receptors. Fluoxetine (Richardson and Roberts, 1991) and L-tryptophan (McGregor et al., submitted) pretreatments significantly decrease the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement. These data are supported by studies reporting a decrease in

the rate of both cocaine and amphetamine intake following pretreatment with either fluoxetine (Leccese and Lyness, 1984; Yu et al., 1986; Porrino et al., 1988; Carroll et al., 1990a; Peltier and Schenk, 1991b), dietary L-tryptophan (Smith et al., 1986; Carroll et al., 1990b), systemic L-tryptophan (Leccese and Lyness, 1984) or quipazine (Leccese and Lyness, 1984).

A significant role for 5-HT in cocaine reinforcement is also evident from the receptor binding studies of Ritz et al. (1987). A negative relationship between the reinforcing efficacy of cocaine analogues in self-administration studies and their binding potencies at 5-HT re-uptake sites was revealed (Ritz et al., 1987). These data suggest that 5-HT plays an important, possibly inhibitory role in cocaine reinforcement.

As a result of the above findings, it might be predicted that the blockade of 5-HT receptors would diminish the adverse effect of 5-HT, thereby increasing the reinforcing efficacy of cocaine. The findings of this study do not support this hypothesis. Instead, we found that the relatively specific blockade of 5-HT receptor subtypes does not alter cocaine's reinforcing properties. Our findings are consistent with those of three studies which report the inability of cinanserin, ritanserin (two 5-HT₂ receptor antagonists) or GR23032F (a 5-HT₂ receptor antagonist) pretreatments to alter the rate of cocaine self-

administration in rats (Porrino et al., 1988; Peltier and Schenk, 1991a,b).

Currently, three major subtypes of 5-HT receptors have been identified: 5-HT₁, 5-HT₂ and 5-HT₃ receptors (Bradley et al., 1986; Frazer et al., 1990; Bonate, 1991).¹ In this thesis, we attempted to examine the effect of individually blocking each of the major 5-HT receptor subtypes on cocaine reinforcement. Unfortunately, since no selective antagonist exists for the 5-HT₁ receptor, methysergide was employed as an aselective 5-HT₁/5-HT₂ receptor antagonist. The effects of 5-HT₂ and 5-HT₃ receptor blockade were examined by employing the highly selective receptor antagonists ketanserin and MDL 72222 respectively.

The present study has shown that the acute, relatively specific blockade of each of these 5-HT receptor subtypes fails to influence the reinforcing properties of cocaine. However, it is possible that either the blockade of other, as of yet, unidentified 5-HT receptor subtypes or the simultaneous blockade of all 5-HT receptor subtypes may be required to alter cocaine reinforcement.

Interpretation of the Results

The inability of 5-HT antagonist pretreatments to alter cocaine self-administration breaking points may have been

¹For a review of 5-HT receptor classification see Appendix A.

the result of employing an insensitive experimental paradigm. In other words, it might be argued that the PR schedule is insensitive to changes in drug reinforcement. However, the PR schedule used in the present study has been shown to be sensitive to changes in drug dose and to a variety of pharmacological manipulations and neurotoxic lesions (Roberts, 1989; Roberts et al., 1989a,b; Loh and Roberts, 1990; Richardson and Roberts, 1991). Furthermore, unlike rate, breaking points derived from the PR schedule appear to be sensitive to 5,7-DHT lesions of the MFB and amygdala (Loh and Roberts, 1990), 6-OHDA lesions of the nucleus accumbens (Roberts, 1989) and hormonal changes across the estrous cycle (Roberts et al., 1989a).

Although 5-HT antagonist pretreatments failed to alter the breaking points reached by rats self-administering cocaine on a PR schedule of reinforcement, it is possible that other aspects of their behaviour may have been altered. However, similar cumulative response patterns of rats self-administering cocaine on a PR schedule following either 5-HT antagonist or vehicle pretreatments have been found (Methysergide data is shown in Figure 4. Data for MDL 72222 and ketanserin are not shown). In each case, in addition to breaking points, both the response rate and time of establishing breaking point remained unchanged.

Figure 4: A comparison of the cumulative response records of a rat self-administering cocaine on a PR schedule of reinforcement following either methysergide (5 mg/kg) (top panel) or vehicle (distilled water, 1 ml/kg) (bottom panel) pretreatments. Vertical increments represent lever responses. Arrows indicate the time of cocaine infusions. Infusions are regularly spaced and each is followed by a consistent post-infusion pause. In each case, a breaking point of 17 was reached which corresponds to a final ratio of 145.

Given that the PR schedule is capable of detecting changes in cocaine reinforcement following a variety of experimental manipulations, there are several alternative factors which may account for the failure of 5-HT antagonist pretreatments to significantly alter cocaine self-administration in the present study. For example, there are several possible reasons why 5-HT lesion data are inconsistent with 5-HT receptor antagonist pretreatment data. First, 5-HT lesions and 5-HT receptor antagonist pretreatments do not inhibit 5-HT function in either the same manner or extent. For example, while 5-HT lesions result in the chronic and non-specific destruction of 5-HT functioning, the 5-HT receptor antagonist pretreatments employed in this study were acute and relatively specific to the 5-HT receptor subtypes. As a result, possibly the chronic rather than the acute blockade of 5-HT receptor subtypes is necessary to alter cocaine's reinforcing properties.

Second, evidence suggests that 5-HT lesions may result in compensatory changes in the DA neurotransmitter system (Simon et al., 1980). It is possible, then, that increases in the motivation to self-administer cocaine following 5-HT lesions may be due to an indirect change in DA function and not on the attenuation of 5-HT function.

Conversely, several studies have reported that 5,7-DHT lesions appear to be specific in depleting 5-HT. For

example, DA and NE concentrations were unaltered following intraventricular injections of 5,7-DHT (Lyness et al., 1980) and 5,7-DHT lesions of the MFB (Leccese and Lyness, 1984) whereas levels of 5-HT were significantly reduced. Loh and Roberts (1990) also reported significant decreases in 5-HT and its metabolite 5-HIAA following 5,7-DHT lesions of either the MFB or amygdala while DA and DOPAC levels remained unchanged.

Alternatively, the failure to find a significant effect of 5-HT antagonist pretreatments on cocaine self-administration behaviour may be the result of employing insufficient doses. However, this seems unlikely since similar doses to those used in this study have been employed successfully in other behavioral studies (Silveira Filho and Graeff, 1977; Leccese and Lyness, 1984; Cunningham and Appel, 1987; Shearman and Tolcsvai, 1987; Gold and Koob, 1988; Carboni et al., 1989; Bilsky and Reid, 1991).

Significant behavioral effects of MDL 72222 have been reported with doses as low as 30 ug/kg although other studies have used doses as high as 1 mg/kg (Higgins et al., 1992). For example, MDL 72222 (30 ug/kg) has been effective in blocking morphine-, nicotine- and MDMA-induced place preference (Aguas et al., 1988; Carboni et al., 1989; Bilsky and Reid, 1991). At this relatively low dose, MDL 72222 was also successful in reducing drug-induced place aversion

elicited by naloxone, picrotoxin and PCP (Aguas et al., 1990).

In this thesis, we examined the effect of MDL 72222 at doses ranging from 15 - 1920 ug/kg and although tested at twice the highest dose previously used, MDL 72222 was without effect.

Ketanserin has been shown to produce significant behavioral effects at a dose of 3.2 mg/kg. Specifically, a significant reduction in LSD self-administration behaviour has been found following pretreatment with 3.2 mg/kg of ketanserin while even lower doses of the drug (0.2 - 1.6 mg/kg), dose-dependently reduced lever responding for LSD by blocking the LSD cue (Cunningham and Appel, 1987). Similar doses have also been found to attenuate the behavioral side effects induced by both L-5-HTP and 5-Me-ODMT pretreatments (Shearman and Tolcsvai, 1987).

In the present study, we tested ketanserin at doses ranging from 0.4 - 6.4 mg/kg. Although the effects of ketanserin were examined at twice the dose reported to significantly block the LSD cue, no significant effect of ketanserin in cocaine reinforcement was found.

Studies have reported significant behavioral effects of methysergide at doses as low as 2.5 - 10 mg/kg. For example, at this dose range, methysergide significantly augments MDMA-induced locomotor hyperactivity (Gold and Koob, 1988). Similarly, at a dose of 10 mg/kg, methysergide significantly

attenuates amphetamine self-administration (Leccese and Lyness, 1984) and potentiates the rate increasing effect of amphetamine on self-stimulation behaviour (Silveira Filho and Graeff, 1977).

We examined the effects of methysergide at doses ranging from 2.5 - 20 mg/kg. Although this is twice the highest dose reported by Silveira Filho and Graeff (1977) to have significant behavioral effects, methysergide failed to significantly alter cocaine reinforcement from baseline levels. Furthermore, in the literature it has been documented that doses of methysergide exceeding 30 mg/kg produce both disruptions in behaviour and motor impairment (Silveira Filho and Graeff, 1977). As a result, in this study doses of methysergide up to 20 mg/kg were tested.

In addition, the inability to find a significant change in cocaine self-administration behaviour following pretreatment with the 5-HT receptor antagonists may have resulted from using inappropriate drug pretreatment intervals. The injection times employed in this study have been based on those used successfully in other behavioral studies (Leccese and Lyness, 1984; Cunningham and Appel, 1987; Carboni et al., 1989; Bilsky and Reid, 1991). We acknowledge the fact that in our experiments the breaking point is not established for approximately 2 hours into the self-administration session. Therefore, it is possible that the time course of drug actions for the three 5-HT receptor

antagonists tested were ineffective within this experimental paradigm.

In summary, the findings of the present study suggest that the relatively specific, acute blockade of 5-HT₁, 5-HT₂, or 5-HT₃ receptor subtypes does not influence the reinforcing effects of cocaine. Thus, possibly either the blockade of other 5-HT receptor subtypes, the simultaneous blockade of all 5-HT receptor subtypes or the chronic blockade of one or more 5-HT receptor subtypes may be required to alter cocaine reinforcement.

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APPENDIX A

Classification of 5-HT Receptors

There currently exists two classification schemes for 5-HT receptors; the ligand binding affinity scheme proposed by Bradley et al., (1986) and the molecular scheme developed by Hartig (1989). Although the Bradley scheme is presently used by researchers, recent advances in the molecular biology of receptors have led to the development of an alternative model of 5-HT receptor classification.

Ligand Binding Affinity Scheme

5-HT receptors are presently classified into four distinct subtypes: 5-HT₁, 5-HT₂, 5-HT₃, and 5-HT₄ receptors. 5-HT₁ receptors are further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes respectively (Bradley et al., 1986; Frazer et al., 1990; Bonate, 1991).

This classification scheme is based on the binding affinity of agonists and antagonists for 5-HT receptor sites. For example, all 5-HT₁ receptors exhibit a high affinity for 5-HT. Subtypes of the 5-HT₁ receptor are defined and thus differentiated from one another on the basis of their diverse affinities for antagonist compounds. In addition, 5-HT₂ and 5-HT₃ receptors are defined by their

low affinity for 5-HT along with a high affinity for selective antagonists (Hartig, 1989).

Most recently, 5-HT₄ receptors have been identified as sites antagonized by high concentrations of ICS 205-930. Little is known about 5-HT₄ receptors since at the present time, no selective ligands for this receptor exist (Clark et al., 1989; Dumuis et al., 1989; Bonate, 1991).

Molecular Scheme

With the advent of molecular biological techniques in the late 1970s, a great deal of additional information on 5-HT receptors became available. On the basis of such findings, an alternative classification scheme for 5-HT receptors was developed (Hartig, 1989).

In the molecular classification scheme, 5-HT receptors are classified into one of two superfamilies. The receptors are then further divided into classes based on degree of structural homology which interestingly enough, corresponds to the second messenger system utilized by the receptors.

Specifically, 5-HT₁ and 5-HT₂ receptors belong to the G protein receptor superfamily. This superfamily contains two classes. For example, since 5-HT_{1C} and 5-HT₂ receptors share 78% of their amino acids and are both coupled to the phosphoinositide second messenger system, they are placed in the phosphoinositide coupled class. The 5-HT_{1A} receptor is

more distantly related to both the 5-HT_{1C} and 5-HT₂ receptors so it is placed in the adenylate cyclase coupled class. On the other hand, the 5-HT₃ receptor is a member of the ligand-gated ion channel superfamily since it is directly coupled to the ion channel opening (Derkach et al., 1989; Hartig, 1989).

Finally, although studies have shown that 5-HT_{1B}, 5-HT_{1D} and 5-HT₄ receptors are coupled to the adenylate cyclase second messenger system, amino acid sequencing data must first be obtained before their place in this classification scheme can be determined (Clark et al., 1989; Dumuis et al., 1989; Bonate, 1990). A comparison of both the ligand binding affinity and molecular classification schemes is given in Figure 5.²

² A detailed account of 5-HT receptor classification is beyond the scope of this thesis but can be found in five recently published review articles: Hartig, 1989; Frazer et al., 1990; Weinstein and Osman, 1990; Bonate, 1991; Hartig et al., 1991).

Figure 5: A comparison of 5-HT receptor classification schemes based on ligand binding affinities and molecular structure. Adapted from Hartig (1989).

LIGAND BINDING AFFINITY SCHEME	MOLECULAR SCHEME
5-HT ₁ (eg) Methysergide 5-HT _{1A} 5-HT _{1B} 5-HT _{1C} 5-HT _{1D}	Adenylate cyclase coupled (G Protein) 5-HT _{1A}
5-HT ₂ (eg) Ketanserin	Phosphoinositide coupled (G Protein) 5-HT _{1C} 5-HT ₂
5-HT ₃ (eg) MDL 72222	Ligand-gated ion channel 5-HT ₃
	5-HT _{1B} , 5-HT _{1D} , 5-HT ₄ ?

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