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BRIEF COMMUNICATION

Evaluation of the Role of Norepinephrine in the Reinforcing Effects of Psychomotor Stimulants in Rhesus Monkeys[1]

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WOOLVERTON, W. L. *Evaluation of the role of norepinephrine in the reinforcing effects of psychomotor stimulants in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **26**(4) 835–839, 1987.—Rhesus monkeys were surgically prepared with intravenous catheters and allowed to self-administer cocaine (0.03–0.1 mg/kg/injection) under a fixed-ratio 10 schedule of drug delivery during daily 2-hour experimental sessions. When responding was stable for cocaine, saline or various doses of nisoxetine, a selective norepinephrine (NE) reuptake blocker, was substituted for cocaine for 5–7 consecutive sessions. Nisoxetine failed to maintain self-administration responding at any dose in 3 of 4 monkeys tested. Pre-session administration of the selective alpha$_1$ NE receptor blocker prazosin (0.2–1.6 mg/kg, IV, 15 minutes pre-session) did not systematically alter cocaine self-administration in any monkey. The results are in contrast to what has been found with DA agonists and antagonists and are consistent with the belief that NE does not play a primary role in the reinforcing properties of psychomotor stimulants.

Norepinephrine Psychomotor stimulants Rhesus monkeys Reinforcing effects

MANY psychomotor stimulants are known to increase the concentration of catecholamines in central nervous system (CNS) synapses. There has been considerable interest in whether these changes in the CNS biochemistry play a role in the reinforcing effects of these compounds. A number of different approaches have been used to investigate this question, including self-administration studies with drugs that are agonists at catecholaminergic receptors as well as pretreatment studies with various antagonists. In general, the conclusions of these studies have been that CNS dopamine (DA) is involved in the reinforcing effects of this class of drugs. Direct and indirect DA agonists can function as positive reinforcers [21,25] and in many cases DA antagonists have been found to increase psychomotor stimulant self-administration in a manner that was consistent with a reduction in unit dose, i.e., antagonism [2, 12, 19, 23, 24]. On the other hand, comparable studies with NE agonists and antagonists have suggested that an increase in the concentration of norepinephrine (NE) in CNS synapses does not play a primary role in the reinforcing effects of psychomotor stimulants. Dogs failed to self-administer the NE agonist methoxamine [6] and the NE antagonists phentolamine, phenoxybenzamine and propranolol either did not alter or decreased psychomotor stimulant self-administration [2, 4, 6, 13, 23, 24].

The purpose of the present study was to further examine the role of NE in the reinforcing properties of psychomotor stimulants in rhesus monkeys. Rhesus monkeys have been found to self-administer DA agonists [21] and DA antagonists can increase the rate of self-administration of psychomotor stimulants [12, 17, 19]. However, with the exception of clonidine [22], drugs with agonist effects specific to CNS NE systems have not been examined for reinforcing properties in this species. Moreover, pretreatment studies in this species with NE antagonists have been limited to phenoxybenzamine and phentolamine, antagonists that have effects at both alpha$_1$ and alpha$_2$ receptors and only penetrate the bloodbrain barrier to a limited extent [13]. In the present experiment, the reinforcing properties of the selective NE reuptake blocker nisoxetine [15,16] were examined in rehsus monkeys experienced in cocaine self-administration. In addition, animals allowed to self-administer cocaine were pretreated with the selective alpha$_1$ antagonist prazosin [11]. The results provide additional support for the belief that NE

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is not the primary mediator of the reinforcing effects of psychomotor stimulants.

METHOD

Animals and Apparatus

The subjects were 4 rhesus monkeys, 2 males and 2 females. The males weighed 8.2 (1034) and 7.8 (4007) kg and the females weighed 4.2 (3033) and 4.3 (3015) kg at the beginning of the experiment. Three monkeys (1034, 3015, 3033) had previously been in studies of IV self-administration of DA receptor agonists and cocaine which included pretreatment with DA antagonists. Monkey 4007 was experimentally naive. Each was fitted with a stainless-steel restraint harness and spring arm which attached to the rear of an experimental chamber (70 cm wide × 84 cm deep × 91 cm high) in which the monkey lived for the duration of the experiment. Each chamber had a Plexiglas window on the front wall that allowed the monkey visual access to the laboratory at all times except during experimental sessions. Water was available continuously and each monkey received 100 to 150 g/day of Purina Monkey Chow after the session. A multiple vitamin supplement in the form of a chewable tablet was provided 3 days/week.

Two response levers (BRS/LVE, PRL-001, Beltsville, MD) were mounted on the inside front of each experimental chamber 10 cm above the floor and a food dish was mounted between them. Four jewelled stimulus lights, two red and two white, were mounted directly above each lever. In addition, two 15 W houselights, one white and one red, were mounted on the ceiling of the cubicle and covered with translucent Plexiglas. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer Co., Chicago, IL). All programming and recording of experimental events was accomplished by solid state equipment located in an adjacent room.

HCl Procedure

Catheters had been implanted previously as follows. Each animal was removed from the chamber and injected with a combination of phencyclidine HCl (1.0 mg/kg IM) and atropine sulfate (0.04 mg/kg IM) followed in 20 to 30 minutes by sodium pentobarbital (10–30 mg/kg IV). When anesthesia was adequate, a silicone catheter (0.08 cm inside diameter, Ronsil Rubber Products, Belle Mead, NJ) was surgically implanted in a major vein. After surgery the monkey was returned to the experimental chamber. The catheter was threaded through the spring arm, out the back of the chamber, and connected to the infusion pump. If a catheter became nonfunctional during the experiment, a new catheter was implanted as before after a 1- to 2-week period to allow any infection to subside.

Daily 2-hr experimental sessions were signalled by the illumination of the white lights. Each animal had previously been trained to press the right lever under a fixed-ratio (FR) 10 schedule for a 10-sec injection of 0.1 mg/kg of cocaine HCl. During an injection the white lights were extinguished and the red house light and lever lights were illuminated. Responses occurring on the left lever were counted but had no other programmed consequences. In the present experiment the animals were allowed to self-administer cocaine at a dose of 0.03, 0.06 or 0.1 mg/kg/injection.

Nisoxetine self-administration was studied in a standard substitution paradigm. After the establishment of stable rates of responding under baseline conditions (less than 10% variation in total number of injections per session for at least 3 consecutive sessions), 0.9% saline was substituted until responding declined to low, stable levels (5–7 sessions). Subsequently, the animal was returned to baseline conditions. When baseline responding was again stable for at least 2 consecutive sessions that approximated previous levels, a dose of nisoxetine was substituted for the same number of sessions that had been required for responding to decline to low levels when saline was available. At least 4 doses of nisoxetine were substituted for cocaine in each monkey in a mixed order using this procedure with baseline conditions reinstated between doses. Doses were tested over an 8-fold range (0.07–0.6 mg/kg/kg/injection) up to a dose which suppressed lever pressing during the first session of the substitution period.

The effects of prazosin on cocaine self-administration were determined in the manner described previously for DA antagonists [19]. When responding was stable under baseline conditions (less than 10% variation in the number of injections/session for at least 3 consecutive sessions), test sessions were begun. A test session consisted of a pretreatment with prazosin (15 minutes pre-session) injected into the catheter followed by enough 0.9% saline to flush the drug into the animal (3–5 ml). The catheter was refilled with cocaine immediately before the session began. Prazosin was administered no more frequently than every fourth day with the additional condition that responding was stable for at least 2 consecutive baseline sessions preceding the pretreatment. Doses were tested twice and ranged between one that had no effect on responding and one that either altered responding or produced visible behavioral effects. Drug doses were tested in a random order in each monkey. Since all available veins had been used in one monkey, prazosin was only tested in 3 subjects.

Data Analysis

Nisoxetine self-administration was compared to responding for cocaine and saline in 2 ways. First, the mean number of injections of nisoxetine over the last 3 sessions of a substitution period was compared to the last 3 sessions of saline availability and to the mean number of injections of cocaine taken in baseline sessions. A dose of nisoxetine was considered to be self-administered if this value was higher than the saline value and the ranges did not overlap. Second, the number of injections in each $^1/_2$-hr of the 2-hr session was recorded and the distribution of injections over the session was calculated and compared for all conditions.

Prazosin effects on cocaine self-administration are presented as a percent of control using the mean injections/session of the 2 baseline sessions immediately preceding each pretreatment as the control value. The effects of saline pretreatment were also determined in each animal (2–8 determinations) and the mean and the 95% confidence limits were calculated from values for these sessions. Drug effects were examined for the first $^1/_2$-hr, 1-hr and the entire 2-hr session. Since there was no systematic variation in these effects, data are presented for the entire session.

Drugs

Nisoxetine HCl was a gift from Eli Lilly Co. (Indianapolis, IN) and prazosin HCl was provided by Pfizer Inc. (Groton, CT). Cocaine HCl was provided by the National Institute on Drug Abuse. Cocaine and nisoxetine were dissolved in 0.9% saline in a concentration appropriate to a

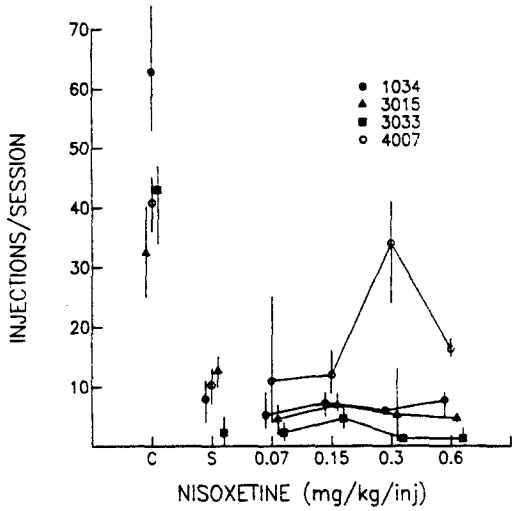

FIG. 1. Nisoxetine self-administration in rhesus monkeys. Each point represents the mean number of injections/session over the last 3 sessions of availability of each dose of nisoxetine or saline (S) for individual monkeys. The points above C represent the self-administration of cocaine (1034, 0.03 mg/kg/injection; 3015, 3033, 0.1 mg/kg/injection; 4007, 0.06 mg/kg/injection) during baseline sessions immediately preceding each dose of nisoxetine (N=6–12 sessions). Vertical lines are the range.

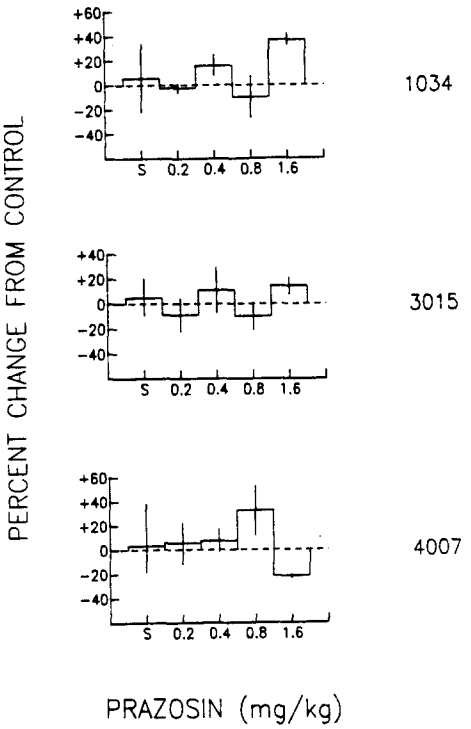

FIG. 3. The effects of prazosin on cocaine self-administration. Various doses of prazosin, indicated on the abscissas, were given IV 15 minutes before the session. Cocaine doses were: 1034, 0.03 mg/kg/injection; 3015, 0.1 mg/kg/injection; 4007, 0.06 mg/kg/injection. Each bar of a histogram represents the percent deviation from control defined as the mean number of injections self-administered in the 2 sessions immediately preceding each prazosin dose. The variability measure for saline (S) is the 95% confidence limits for those monkeys that had multiple saline tests (1034, 3015) and the range of 2 tests for 4007. The variability measures for the prazosin data are the range of 2 tests.

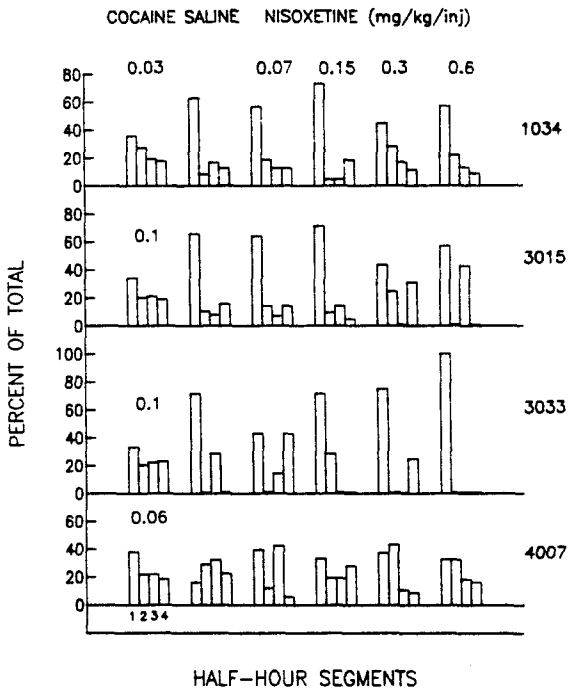

FIG. 2. The distribution of responding over the session for cocaine, saline and various doses of nisoxetine. Doses (mg/kg/injection) are indicated above the histograms. Each bar of a histogram represents the percent of the total injections/session that were taken in successive $^1/_2$-hr segments of the 2-hr session. The last 3 days of saline and nisoxetine availability were used in the calculations while cocaine data were averaged for baseline sessions over the entire experiment.

1.0 ml injection. Prazosin was prepared in sterile water at a concentration of 1.0 mg/ml and injection volume was adjusted to give the proper dose. All doses refer to the salt.

RESULTS

All monkeys self-administered cocaine during baseline sessions (Fig. 1). Mean self-administration rates for cocaine ranged between 32 (3015) and 63 (1034) injections/session. When saline was substituted for cocaine responding declined to approximately 10 injections/session over a period of 5–7 sessions. Three of the four monkeys failed to self-administer nisoxetine above saline levels at any dose tested. In these 3 monkeys, the first session of exposure to 0.3 or 0.6 mg/kg nisoxetine resulted in suppression of responding relative to that seen during the first session of saline self-administration, indicating that behaviorally active doses had been achieved (data not shown). In addition, in these sessions when intake was high (6–24 mg/kg/session) because of high unit doses, behavioral effects such as agitation and tremors were apparent. The fourth monkey (4007) self-administered nisoxetine above saline levels at 2 doses, 0.3 and 0.6 mg/kg/injection. At these doses, rate of self-administration was inversely related to dose. This monkey also exhibited behavioral effects of nisoxetine.

Self-administration of cocaine was relatively evenly distributed over the session with 52–64% of the injections taken in the first $^1/_2$ of the session (Fig. 2). In contrast, responding for saline was concentrated in early segments of the session.

In 3 of the monkeys more than 60% of the saline injections were taken in the first 1/4 of the session. Responding for saline was more evenly distributed in monkey 4007. The pattern of responding for nisoxetine was similar, in most cases, to that seen with saline, regardless of the dose.

Prazosin failed to consistently alter cocaine self-administration up to a dose of 1.6 mg/kg (Fig. 3). For monkey 1034, 1.6 mg/kg prazosin increased cocaine self-administration to 142% of control values in the first test but the results of the second test were within the 95% confidence limits for saline pretreatments. Similarly, the first test of 0.8 mg/kg prazosin in 4007 had no effect on cocaine self-administration while self-administration was increased (to 153% of control) following the second pretreatment with this dose of prazosin. The highest dose of prazosin (1.6 mg/kg) decreased responding in 4007. The effects of all other prazosin injections fell within the 95% confidence limits of saline effects. Following 1.6 mg/kg of prazosin, observable effects included ptosis and sedation.

DISCUSSION

In 3 of 4 rhesus monkeys tested, the indirect NE agonist nisoxetine failed to maintain IV self-administration above the levels found with saline and the pattern of responding for nisoxetine was similar to the pattern of responding for saline. These results are consistent with the findings of Risner and Jones [6] that dogs do not self-administer the direct NE agonist methoxamine. In contrast, both direct and indirect DA agonists functioned as positive reinforcers in rhesus monkeys [5, 14, 21]. That sufficiently high doses of nisoxetine were tested is suggested by experimental observations of response rate reductions at high doses of nisoxetine and of observable behavioral effects in the monkeys. In addition, a dose of 4.8 mg/kg substituted for *d*-amphetamine as a discriminative stimulus in rhesus monkeys [18], a dose that was often exceeded in the present self-administration study.

Although it is unclear why nisoxetine was self-administered by a fourth monkey (4007), it is possible that pre-existing individual differences played a role. However, it should also be noted that this was the only monkey that was experimentally naive at the beginning of the study; the others had extensive experience with self-administration of cocaine, direct DA agonists and pretreatment with DA antagonists [19,21]. It is possible that some aspect of this pharmacological and/or behavioral history played a role in this difference.

In a second experiment, the alpha$_1$ NE antagonist prazosin failed to alter the self-administration of cocaine. It was clear that sufficiently high doses of prazosin were tested since grossly observable behavioral effects were noted. These results are consistent with the findings of others that phentolamine and phenoxybenzamine do not alter self-administration of psychomotor stimulants [2, 6, 13]. In contrast, DA antagonists can increase cocaine self-administration at some doses [17,24]. Together with the lack of self-administration of nisoxetine, the results suggest that central NE actions of psychomotor stimulants do not play a primary role in their reinforcing effects in rhesus monkeys.

Nisoxetine has also been examined behaviorally in animals trained to discriminate a psychomotor stimulant from saline. In mice, pigeons and rhesus monkeys, nisoxetine completely substituted for *d*-amphetamine in this paradigm [3, 9, 18]. In addition, prazosin has been found to block the discriminative stimulus properties of *d*-amphetamine and nisoxetine in mice [10]. That is, in a paradigm felt by many to represent an animal model of subjective effects [8], nisoxetine has clear amphetamine-like effects and prazosin can block these effects. The reason(s) why a compound that apparently produces an amphetamine-like discriminative stimulus fails to function as a positive reinforcer is unclear. One possibility suggested by the data is that *d*-amphetamine discrimination is based primarily upon NE actions in the CNS while its reinforcing effects involve DA. However, previous research with rats has also suggested a role for DA in the discriminative stimulus properties of *d*-amphetamine [1, 7, 20]. It is possible that species differences exist, that is, that these effects of *d*-amphetamine are predominantly dopaminergic in nature in rats only. Regardless of the account of these apparent discrepancies they do reflect the fact that self-administration and drug discrimination paradigms may measure different aspects of drug action and that there are components of the *d*-amphetamine discriminative stimulus other than those that contribute to its self-administration. Moreover, the predictions of the two experimental paradigms regarding the dependence potential of nisoxetine would be quite different.

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