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Mediation in the Nucleus Accumbens of the Discriminative Stimulus Produced by Cocaine

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WOOD, D. M. AND M. W. EMMETT-OGLESBY. *Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine*. PHARMACOL BIOCHEM BEHAV 33(2) 453–457, 1989.—Rats were trained to detect an intraperitoneal (IP) administration of cocaine, 10.0 mg/kg, using a two-lever choice discrimination procedure in which food reinforcement was only delivered following 10 responses on the correct lever (FR10): one lever was correct after cocaine injection, and the other lever was correct after saline injection. Following training, cocaine was generalized to the cocaine training stimulus in a dose-dependent manner. Subsequently, guide cannulae were implanted bilaterally in the prefrontal cortex (from bregma, A = 2.7, L = 1.0, V = 3.0 mm), nucleus accumbens (A = 2.2, L = 1.5, V = 6 mm) or caudate putamen (A = 0.2, L = 2.5, V = 4). Injections were made via cannulae that extended 1 mm past the tip of the guide cannulae. Injection in the nucleus accumbens substituted for the IP training dose of cocaine in a dose-dependent manner with maximum generalization occurring with 10 μg of cocaine per side (87% cocaine-lever responding); in contrast, injections of cocaine in the prefrontal cortex or caudate-putamen produced only partial cocaine-lever responding (a maximum of 48 and 37% cocaine-lever responding, respectively). These data support the hypothesis of central mediation of the cocaine stimulus and show that cocaine administered in the nucleus accumbens is sufficient to produce the stimulus. The partial substitution of cocaine in the prefrontal cortex and caudate-putamen may reflect partial mediation of the cocaine stimulus in these brain areas.

Cocaine Nucleus accumbens Medial prefrontal cortex Caudate-putamen Discrimination stimulus Rat Cannula

IN drug discrimination procedures, by differentially reinforcing separate behaviors, subjects can be trained to emit one behavior after treatment with a drug, and to emit a different behavior after treatment with a drug-vehicle. For example, rats can be trained to discriminate cocaine from saline using an operant procedure, where responding on one lever is reinforced with food pellets only following injection with cocaine, and responding on a second lever is reinforced with food only following injection with saline. Experiments using discrimination procedures have established that animals and humans detect the stimulus properties of drugs in parallel (2–4, 11, 20). Thus, the drug discrimination procedure in animals provides an opportunity for investigating neurobiological mechanisms that may mediate the subjective effects of drugs of abuse in humans.

With regard to cocaine, several experiments have provided evidence for the role of dopamine in mediating the discriminative stimulus properties of this drug. For example, dopamine receptor agonists such as apomorphine substitute for the cocaine stimulus (5, 15, 24), and dopamine receptor antagonists block the cocaine cue (6, 7, 13–15). In addition, the stimulus produced by cocaine is mediated in the central nervous system: cocaine administered into the lateral ventricles (ICV) substitutes for cocaine trained by IP administration (23), and cocaine given ICV is approximately 40 times more potent in producing the stimulus than cocaine given IP. This increased potency is most easily accounted for by assuming that cocaine administered ICV is limited only by local diffusion for interaction with receptors in various brain areas, whereas with IP administration of cocaine, the amount of drug reaching brain receptors is dependent on the absorption, distribution, and metabolism of cocaine peripherally.

Although these experiments suggest that the cocaine stimulus is mediated at least in part through brain dopaminergic mechanisms, we are aware of only a single abstract showing an involvement of specific brain areas in the mediation of the cocaine stimulus (1). In that study, lesion of the dopaminergic fibers innervating the nucleus accumbens resulted in a shift to the right of the dose-effect curve for the detection of cocaine. In the present experiment, we administered cocaine directly into discrete brain areas rich in dopaminergic nerve terminals and determined whether these injections would substitute for cocaine trained as a discriminative stimulus by IP injection. As our sites of injection, we chose the caudate-putamen, the medial prefrontal cortex, and the nucleus accumbens. These regions were selected because they have been implicated as critical in mediating one or another behavioral

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effects of cocaine. For example, cocaine produces a variety of stereotypies in rats (19), and these effects can be mimicked by injecting dopaminergic agonists into the caudate-putamen (7). Perhaps of more importance with regard to the subjective effects of cocaine, Goeders and Smith (12) have reported that rats will self-administer picomole quantities of cocaine into the medial prefrontal cortex, suggesting that this area is important for mediating the reinforcing properties of cocaine. Although the relationship between the discriminative stimulus properties and the reinforcing effects of cocaine are unknown, we were interested in determining whether a site mediating one of these effects would also mediate the other. Finally, cocaine and *d*-amphetamine have comparable stimulus properties (10,22), and Nielsen and Scheel-Kruger (16) reported that *d*-amphetamine administered directly into the nucleus accumbens generalized to the stimulus properties of *d*-amphetamine trained by IP injection. Their results suggest that the nucleus accumbens may also be important in mediating the discriminative stimulus properties of cocaine.

METHOD

Subjects and Apparatus

Thirty male rats of the Long-Evans strain (Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 320 ± 10 g were subjects. Discrimination training was conducted in standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA). Each chamber contained two levers, one on either side and equidistant from a food cup. Scheduling of reinforcement contingencies and recording of data were done through IBM-PC-compatible microcomputers and printers connected to the chambers through LVB interfaces (Med Associates, Inc.) using a program described by Spencer and Emmett-Oglesby (21).

Drugs

Cocaine hydrochloride was obtained from Mallinckrodt Inc. (St. Louis, MO) and was dissolved in 0.9% saline. The route of drug administration during training was intraperitoneal (IP).

Discrimination Training

Rats were trained to press a lever, and their behavior was shaped progressively until 10 lever-press responses (FR10) were required to obtain food reinforcement. Subsequently, subjects were trained to press one of the levers following cocaine (10 mg/kg) administration, and the other lever following 0.9% saline. For this training, cocaine or saline was injected 15 min prior to each 10 min session. There were equal numbers of cocaine and saline training sessions, which were presented in an irregular sequence so that no condition occurred in more than three successive training sessions. No cue other than the the effect of the drug was available to guide appropriate lever selection. For complete shaping and training procedure see Wood and Emmett-Oglesby (22). Discriminative control was defined as 10 successive sessions of correct-lever responding in which, at the start of each session, when saline was injected, 10 responses were emitted on the saline lever with fewer than 10 responses on the cocaine lever, and when cocaine was injected, 10 responses occurred on the cocaine lever with fewer than 10 responses on the saline lever. Once this criterion had been achieved, tests were conducted whenever the correct lever was selected for four consecutive sessions.

Cannulation Procedure

Rats were cannulated bilaterally in specific brain sites. Bilateral guide cannulae assemblies were made from No. 26 gauge stainless steel hypodermic tubing and 10 mm teflon molds according to the procedure of Czech and Stein (9). Cannulae were implanted under pentobarbital (60 mg/kg) anesthesia using coordinates from Paxinos and Watson (17). Nonperforming animals were implanted with the bilateral cannulae in the nucleus accumbens, prefrontal cortex, and caudate-putamen, and the placements were verified histologically for accuracy of the coordinates before any implants were made in trained rats. From bregma, midline and the dura as reference, coordinates for cannulation were: prefrontal cortex, anterior = 2.7 mm; lateral = 1.0 mm; and ventral = 3.0 mm; nucleus accumbens, A = 2.2 mm; L = 1.5 mm; and V = 6.0 mm; and caudate-putamen, A = 0.2; L = 2.5 mm; and V = 4.0 mm. After recovery from surgery and restabilization on the peripheral detection of cocaine, dose-effect data for the detection of centrally administered cocaine were determined as described in general microinjection testing technique below.

Discrimination Testing

The testing procedure for obtaining both peripheral and central cocaine dose-effect curves was similar to the training procedure, except that 10 responses on either lever produced food reinforcement, and sessions were terminated either after one reinforcement was obtained or 10 min had elapsed. In addition, the subjects were placed in the operant chambers 15 min following peripheral administration and 5 min following central administration. The latency period from drug administration to placement in the operant chambers (5 min) for central administration of cocaine was selected based on our previous investigation [see (23)].

Microinjection technique consisted of using No. 33 gauge cannulae which were inserted inside the guide cannulae. The injection cannulae were attached to No. 22 polyethylene tubing using acrylic glue. The injection cannulae were cut 1 mm longer than the guide cannulae, so that when injection occurred, drug did not diffuse back up the guide cannulae. Once cannulae were inserted, injections were made by hand over a 30-second period using a volume of 0.5 μl per side. Two people assisted in the injection procedure such that bilateral injection was performed simultaneously. For these injections, the No. 22 gauge tubing was connected to a 5 μl Hamilton syringe. Cocaine (5–40 μg) was dissolved in freshly prepared artificial CSF. Each rat received a total of 7 intracerebral injections (3 CSF injections and 4 cocaine injections). At the end of each intracerebral test, stimulus control was verified by analyzing the percentage of drug-lever responding following each subsequent regular training session. In all rats, injections occurred in the order 5, 20, 40, and 10 μg. At least 4 training sessions separated each intracerebral test. At the conclusion of this experiment, rats were sacrificed, and cannula placement was verified histologically.

Data Analysis

The data are presented in terms of percentage of responses on the drug-appropriate lever. Statistical analysis of dose-effect data was tested using repeated measures analysis of variance using a computerized IBM-compatible statistical package, General Univariate and Multivariate Analysis of Variance.

RESULTS

It took approximately 50 sessions of training for rats to discriminate cocaine, 10 mg/kg, from saline, respectively, and to meet the criterion of selecting the correct lever on ten consecutive sessions. The subjects were trained for an additional 20 sessions prior to any dose-effect testing and for an additional 20 sessions

TABLE 1

SUBSTITUTION OF COCAINE FOR THE COCAINE TRAINING STIMULUS*

Brain Site	Cocaine (mg/kg)	% Drug Responding†	N
Nucleus Accumbens	2.5	39%	8
	5.0	73%	8
	10.0	100%	8
Caudate-Putamen	2.5	25%	8
	5.0	61%	8
	10.0	89%	8
Prefrontal Cortex	2.5	31%	6
	5.0	68%	6
	10.0	100%	6

*Cocaine, 10 mg/kg, was trained by IP injection, and the various doses tested were administered IP. The Brain Site designation shows which groups these subjects were subsequently assigned to for direct CNS injection of cocaine.

†Data are shown as the percent of responses accumulating on the drug lever prior to receiving the first reinforcer.

TABLE 2

STIMULUS CONTROL ACROSS THREE INJECTIONS OF ARTIFICIAL CSF*

Brain Site	CSF Trial	% Drug Responding†	N
Nucleus Accumbens	1	19%	8
	2	15%	8
	3	1%	8
Caudate-Putamen	1	42%	8
	2	14%	8
	3	2%	8
Prefrontal Cortex	1	18%	6
	2	30%	6
	3	15%	6

*Tests were performed 5 min after injection of 0.5 μl of artificial CSF given bilaterally into the indicated brain structures.

†Data are shown as the percent of responses accumulating on the drug lever prior to receiving the first reinforcer.

after cannulation. The rats discriminated cocaine in a dose-dependent fashion following peripheral cocaine administration (Table 1).

Cannulae placements in the specific brain sites were histologically verified. Five rats died either shortly following surgery or prior to execution of the experiment; 3 in the prefrontal cortex; 1 each in the nucleus accumbens and caudate-putamen. Three other rats were removed from the study due to inaccurate cannulae placements. The total number of subjects in each group was 8 in the nucleus accumbens and caudate-putamen and 6 in the prefrontal cortex. In the first test session, intracerebral administration of artificial cerebrospinal fluid (CSF) and/or the restraining procedure produced greater cocaine-appropriate lever responding compared to previous tests with saline. However, by the third consecutive habituation session with artificial CSF, this effect was abolished (Table 2).

Cocaine injected in the nucleus accumbens produced dose-dependent selection of the cocaine-appropriate lever, with 87% of subjects selecting the cocaine lever after 10 μg cocaine injections given simultaneously on both sides of the brain. In contrast, cocaine (5–20 μg) injected into either the caudate-putamen, or prefrontal cortex produced only partial cocaine-appropriate lever responding (Fig. 1). Analysis of variance performed on the percent drug-lever responding for the three cocaine dose-effect curves demonstrated a significant effect of drug-lever responding following injections in the nucleus accumbens, $F(2,19)=4.67$, $p<0.05$. Doses of 40 μg cocaine were behaviorally disruptive, and resulted in no lever selection during the 10-min test session. Data from these tests are not presented. Since the test session was terminated after the first reinforcement (ten lever presses on either lever) rate of responding was not analyzed. However, to determine that lever choice data were actually the result of responding under stimulus control, the average number of lever presses for the test session was recorded for each brain site and dose. Under the concurrent FR10 schedule used for testing, the range of possible number of lever presses was from 10 (10 on the selected lever, with 0 on the nonselected lever) to a maximum of 19 (10 on the selected lever, with 9 on the nonselected lever). The average number of presses for the three brain sites for the lowest dose of cocaine (5 μg) was 10.2 and for the highest dose of cocaine (40 μg) was 11.3. Thus, responding following CNS injection occurred essentially in an all-or-none fashion on the selected lever.

DISCUSSION

Cocaine injected into the nucleus accumbens substituted for cocaine trained by peripheral administration, and the substitution occurred at doses less than 1/100 of that for IP training. Previously we have shown that cocaine given into the ventricles substituted for cocaine given peripherally (23). In addition, in that report, cocaine given ICV was roughly 30–40 times more potent than cocaine given IP, whereas in the present investigation cocaine

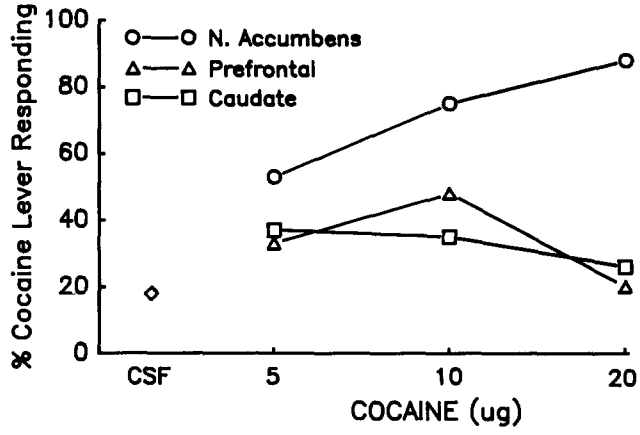

FIG. 1. Substitution of cocaine injected directly into various brain areas for cocaine trained as a discriminative stimulus by IP administration. Cocaine, 10 mg/kg, given IP 15 min prior to placing in the operant chamber, was trained as a discriminative stimulus. Following acquisition of the discrimination, rats were implanted bilaterally with cannulae in either the nucleus accumbens, the medial prefrontal cortex, or the caudate-putamen. Subjects were stabilized to the injection of artificial CSF, and subsequently they were tested for the detection of cocaine at the doses shown. Abscissa: dose of cocaine tested, where one-half of the indicated concentration was injected in a volume of 0.5 μl on each side of the brain. Ordinate: percent of responses occurring on the drug lever prior to the occurrence of reinforcement.

given into the accumbens was approximately 150 times more potent than cocaine given IP. This increase in potency from the ventricles to the accumbens is consistent with the hypothesis that the discrimination of cocaine is mediated, at least in part, in the nucleus accumbens.

In contrast to the positive results found in the nucleus accumbens, cocaine administered into either the medial prefrontal cortex or caudate-putamen produced only partial cocaine-appropriate lever responding. These data suggest that the nucleus accumbens plays a major role in mediating the cocaine discrimination, and that these other structures are likely to be less important in this regard. The conclusion that the nucleus accumbens is an important structure in the mediation of the cocaine discrimination is supported by the observation that 6-hydroxydopamine lesions of the nucleus accumbens attenuate the cocaine discriminative stimulus (1); interestingly, lesions of the nucleus accumbens have also been reported to decrease the self-administration of cocaine (18). In addition, *d*-amphetamine substitutes for cocaine (22), and *d*-amphetamine injected into the nucleus accumbens will substitute for *d*-amphetamine trained by IP injection (16). Moreover, when compared by IP administration, cocaine is approximately ⅛ as potent as *d*-amphetamine (22); in the present study 20 μg of cocaine produced greater than 80% cocaine-lever selection when injected into the nucleus accumbens, and in the Nielsen and Scheel-Kruger (16) study, 5 μg of *d*-amphetamine injected into the nucleus accumbens produced greater than 80% responding on the *d*-amphetamine lever. Although caution is necessary when making comparisons across studies in which different strains of subjects were involved and training doses of amphetamine and cocaine were not explicity equated, it is nonetheless interesting that the potency ratios reported peripherally for these drugs were roughly comparable when they were administered into the accumbens.

Whether the cocaine stimulus is mediated entirely through dopaminergic mechanisms in the nucleus accumbens is unknown; the partial cocaine-responding produced by prefrontal cortex or caudate-putamen administration may indicate that these areas are involved in the cocaine discrimination, but to a lesser extent than the nucleus accumbens. Nielsen and Scheel-Kruger (16) reported that *d*-amphetamine administered into the nucleus accumbens was sufficient, but not necessary, for producing the amphetamine cue; thus, although this structure is important in producing the stimulus properties of psychostimulant drugs, it is not critical.

A role for dopamine in mediating the discriminative stimulus produced by cocaine has been well established (6, 13–15, 24). The data from this experiment are consistent with the hypothesis that the discriminative stimulus produced by cocaine is mediated by the dopaminergic system, in particular, via dopaminergic transmission in the mesolimbic pathway. Since the drug discrimination paradigm has been proposed as an in vivo assessment of the effects of drugs which parallel the subjective effects experienced in humans (20), the drug discrimination procedure offers a powerful methodology for investigating brain mechanisms that may be important in producing subjective effects associated with drugs of abuse.

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