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Discriminative Stimulus Effects of Cocaine: Antagonism by Dopamine D_1 Receptor Blockade in the Amygdala[1]

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CALLAHAN, P. M., S. K. BRYAN AND K. A. CUNNINGHAM. *Discriminative stimulus effects of cocaine: Antagonism by dopamine D_1 receptor blockade in the amygdala.* PHARMACOL BIOCHEM BEHAV **51**(4) 759-766, 1995.—Mesolimbic dopamine (DA) D_1 and D_2 receptors appear to be involved in mediating the discriminative stimulus effects of cocaine. The purpose of the present study was to investigate the role of the amygdala and, in particular, central amygdala DA D_1 receptors, in modulating the stimulus effects of cocaine. Thus, rats were trained to discriminate cocaine (10 mg/kg, IP) from saline using a two-lever, water-reinforced FR 20 drug discrimination task. In substitution tests, systemic (IP) administration of cocaine (0.625–20 mg/kg) produced a dose-related increase in cocaine-lever responding. Intracranial bilateral injections of cocaine (20–200 μg, total dose) into the central amygdala engendered, at best, a partial substitution (<60% drug-lever responding) for the systemic cocaine cue. Central amygdala microinjections of artificial cerebrospinal fluid (ACSF; 1 μl/side) or SCH 23390 (0.5–2 μg, total dose) resulted in primarily saline-appropriate responding. In antagonism tests, bilateral injections of the DA D_1 receptor antagonist SCH 23390 (0.5–2 μg, total dose) into the central amygdala produced a dose-related blockade of a systemic dose of cocaine (5 mg/kg) that engendered >85% cocaine-lever responding when given alone. Additionally, bilateral injection of a fixed dose of SCH 23390 (2 μg) into the central amygdala resulted in a rightward shift in the cocaine dose-response curve (2.5–20 mg/kg). Although administration of cocaine into the central amygdala does not mimic the systemic cocaine cue, the present results demonstrate that DA D_1 receptors located within the central amygdala appear to have a modulatory role upon the discriminative stimulus properties of cocaine.

Cocaine Drug discrimination Central amygdala Dopamine D_1 receptors SCH 23390 Microinjection Rat

MESOACCUMBENS dopamine (DA) systems appear to be critically involved in modulating the discriminative stimulus (7,16,17,30,44) and reinforcing effects (22,35,38) of indirect DA agonists such as amphetamine and cocaine. For example, 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens reduce the reinforcing (25,35) and discriminative stimulus effects of cocaine and amphetamine (16,17). Additionally, intra-accumbens DA D_1 and D_2 receptor antagonists block the reinforcing (4,28,38) and discriminative stimulus effects of stimulants (7,30). Although mesoaccumbens DA systems appear to be a prominent neural substrate for the reward-relevant actions of psychostimulants, it is not the only mesolimbic region involved. Indeed, the medial prefrontal cortex has been demonstrated to maintain cocaine self-administration (20), whereas prefrontal cortex 6-OHDA lesions reduce intracortical cocaine self-administration (21) and increase the sensitivity to cocaine, such that subthreshold doses become reinforcing (39).

Implicated in psychological processes (mood, emotion) and affected by psychoactive drugs, the amygdala may also be an important site of action for cocaine, particularly as it is sensitive to cocaine-induced kindling (33) and receives dense innervation from DA, norepinephrine, and serotonin neurons in the midbrain (19,42). In addition, anatomical (2,23,46), electrophysiological (8,24,45) and behavioral (15,29,37) research has confirmed that a functional interdependence exists between the nucleus accumbens and amygdala, areas that are known to be involved in modulating behaviors related to re-

[1] Some of these data were presented at the annual FASEB Experimental Biology meeting in New Orleans (1993).
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ward and motivation (1,29,34,43). In particular, 6-OHDA lesions of the amygdala have been demonstrated to facilitate self-administration of a low dose of amphetamine (15), whereas ibotenic acid lesions of the sublenticular substantia innominata [a brain region considered to be part of the "extended amygdala" (2)] decreased fixed as well as progressive ratio schedules of reinforcement maintained by cocaine self-administration (37). The importance of the amygdala has been further demonstrated by the finding that intracerebral injection of the DA D_1 receptor antagonist SCH 23390 into the amygdala blocks cocaine self-administration (4,28) as well as cocaine-induced hyperlocomotion (28). Thus, alterations in the behavioral effects induced by psychostimulants may be a direct result of disruption of amygdala-accumbens interconnections.

Because mesoaccumbens DA D_1 and D_2 receptors appear to be involved in modulating the subjective effects of psychostimulants (7,16,30,44) and these DA receptor subtypes are localized within the amygdala (40), the purpose of the present experiment was to further investigate the role of the amygdala and, in particular, central amygdala D_1 receptors in modulating the discriminative stimulus effects of cocaine. Thus, rats were trained to discriminate cocaine (10 mg/kg) from saline using a two-lever, water-reinforced drug discrimination task. Substitution and antagonism tests with cocaine and the DA D_1 receptor antagonist SCH 23390 were conducted following either systemic (intraperitoneal, IP) or intra-amygdala drug administration.

METHOD

Subjects

Experimentally naive, male Sprague-Dawley rats (SASCO, Houston, TX) were housed in pairs in a colony of constant temperature (21–23°C) and humidity (40–50%); lighting was maintained on a 12L : 12D cycle (0700–1900 h). Although food was always available, the water each animal received was limited to the amount that was obtained during training sessions in the operant chambers, after test sessions (10–15 min) and on weekends (24 h).

Apparatus

The apparatus and general procedure have been described in detail elsewhere (5). Briefly, eight two-lever operant chambers (Model 80001; Lafayette Instrument, Lafayette, IN) equipped with a water-filled dispenser mounted equidistant between two response levers on one wall and housed in a light- and sound-attenuating shell (Model 80015; Lafayette Instrument) were used. Illumination was provided by a 28-V house light; ventilation and masking noise were supplied by a blower. A computer was used to program and record all experimental events.

Behavioral Procedures

Rats (N = 20) were trained to discriminate cocaine (10 mg/kg) from an equivalent volume (1 ml/kg) of saline (0.9% NaCl). Drug or saline was administered IP 15 min prior to daily (Monday–Friday) sessions. Initially, training began under a schedule of continuous water reinforcement (FR 1) with only the stimulus-appropriate (drug or saline) lever present ("errorless" training); the schedule of reinforcement was increased until all animals were responding reliably under a fixed ratio schedule for each experimental condition (FR 20). To control for the possible development of position cues based upon olfactory stimuli, a pseudorandom relationship was maintained between the lever programmed to deliver reinforcement for each consecutive subject run in the same experimental chamber (18). After responding stabilized on an FR 20 schedule, both levers were presented simultaneously and rats were required to respond on the stimulus-appropriate (correct) lever to obtain (water) reinforcement; there were no programmed consequences for responding on the incorrect lever ("discrimination" training). This phase of training continued until the performance of all animals attained criterion (individual mean accuracies of at least 80% correct prior to the first reinforcer for 10 consecutive sessions).

Test Procedures

Test sessions were initiated once all animals reached criterion (above) and were conducted one to two times per week in irregular order; cocaine and saline sessions intervened between test sessions to maintain discrimination accuracy. Only rats that met the 80% performance criterion during the preceding cocaine and saline sessions were tested. During test sessions, rats were placed in the chamber as during training sessions and upon completion of 20 responses on either lever or after the session time (20 min) had elapsed, a single (water) reinforcer was delivered, the house light was turned off, and the animals were removed from the chamber. After return to the home cages, all rats were allowed 10 to 15 min of free access to water.

Two pharmcological test manipulations were performed during test sessions. In *substitution* (generalization) tests, all rats (N = 20) were tested for lever selection 15 min after receiving a single systemic injection (IP) of cocaine (0.625–20 mg/kg) or saline. Cocaine dose–response and saline control tests were performed both prior to and after intracranial implants to ensure test reliability. Additionally, all rats (N = 20) received a bilateral intra-amygdala injection of artificial cerebrospinal fluid (ACSF) and were tested for lever selection 15 min later. To mimimize the total number of microinjections each animal received, rats were separated into two experimental groups. Group 1 rats (N = 8) were tested for substitution following bilateral intra-amygdala injections of cocaine (20, 100, 150, and 200 μg, total dose) and a single dose of SCH 23390 (2 μg, total dose) whereas group 2 rats (N = 12) received bilateral intra-amygdala injections of SCH 23390 (0.5, 1, and 2 μg, total dose). Rats were tested for lever selection 15 min after receiving the intra-amygdala injection. In combination (antagonism) tests, group 1 rats were tested for lever selection following intra-amygdala administration of the DA D_1 receptor antagonist SCH 23390 (0.5, 1, and 2 μg) prior to a systemic (IP) dose of cocaine (5 mg/kg), which produced >85% cocaine-lever responding when given alone. SCH 23390 was administered 45 min prior to test and cocaine (5 mg/kg) was administered 30 min before test. In group 2 rats, the ability of a fixed dose of SCH 23390 (2 μg) infused into the central amygdala to alter various systemic (IP) doses of cocaine (2.5, 5, 10, and 20 mg/kg) was assessed. To mimimize diffusion of the drug away from the injection site, SCH 23390 was administered 5 min prior to administration of cocaine and rats were tested for lever selection 15 min later. An injection of saline followed all central amygdala microinjections as an "injection" control measure. Intra-amygdala doses are expressed as the total bilateral dose administered (μg). Rats received no more than 10 intracerebral injections (three ACSF

injections and seven drug (cocaine or SCH 23390) microinjections.

Surgery and Cannulation Procedures

Bilateral guide cannulae (26 ga) were stereotaxically implanted into the central amygdala (AP = −2.3 mm from bregma, ML = ±4.4 mm, DV = −7.4 mm (31) under pentobarbital (50 mg/kg) anesthesia. Following 5–7 days of recovery, discrimination training and testing was reinstated. Microinjections of ACSF, cocaine, or SCH 23390 were administered through 33-ga internal cannulae that extended 0–2 mm below the guide cannulae tips. The injection volume was 1 μl per side and drug was injected bilaterally over a 1-min (group 1) or 2-min period (group 2) using a Harvard infusion pump (Model 2274); injection cannulae remained in place for an additional 3–5 min to allow for diffusion away from the cannulae tips. At the end of the study, rats were sacrificed, and intra-amygdala cannulae placements were verified histologically (Fig. 1).

Data Analysis

During training sessions, accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of drug-appropriate responses to total responses prior to the delivery of the first reinforcer. Response rates (responses per minute) were also evaluated during training and test sessions as a measure of behavioral disruption. For training sessions, the response rate was calculated as the total number of responses emitted on either lever before completion of the first FR 20 divided by the number of minutes taken to complete the first ratio. During test sessions, the response rate was calculated as the total number of responses prior to the completion of 20 responses on either lever divided by the number of minutes taken to complete that FR 20. Only data from animals that completed the FR 20 during test sessions were used.

For systemic and intra-amygdala substitution tests with saline, cocaine and SCH 23390 administered alone, Student's *t*-test for repeated measures was used to compare cocaine-lever responding and response rate on the immediately preceding cocaine maintenance sessions with performance on test sessions. A one-way analysis of variance (ANOVA) for repeated measures was used to determine main effects of SCH 23390 dose on cocaine-lever responding and response rate; Dunnett's multiple-comparison test was used to compare cocaine alone (5 mg/kg) with each dose of SCH 23390 given in combination with cocaine. A two-way ANOVA for repeated measures was used to assess whether the dose-effect relationship differed in the presence vs. absence of SCH 23390; Student's *t*-test was used to compare each dose of cocaine with and without SCH 23390. All comparisons were made with an experimentwise type I error rate (alpha) set at 0.05. A compound was said to have substituted fully for cocaine if at least 80% of responses occurred on the drug-appropriate lever following at least one dose of that compound; similarly, a complete antagonism was said to occur when no more than 20% drug-appropriate responding occurred after pretreatment with at least one dose of SCH 23390 given in combination with cocaine (5 mg/kg). Log-probit analysis was also used to estimate the dose (milligrams per kilogram) of each agonist predicted to elicit 50% drug-lever responding (ED_{50}) and each antagonist to decrease drug-lever responding by 50% [AD_{50} (41)].

Drugs

Although the order of drugs and doses tested was irregular over time, testing order was the same in all animals. Doses of all drugs refer to the weight of the salt. Cocaine HCl (National Institute on Drug Abuse, Bethesda, ML) and SCH 23390 maleate (Schering-Plough Corp., Bloomfield, NJ) were prepared in physiological saline (0.9% NaCl) and injected systemically in a volume of 1 ml/kg, whereas drugs given centrally were prepared in ACSF and injected in a 1-μl vol. The ACSF solution contained (in mM): NaCl, 122; KCl, 3.1; NaH_2PO_4, 0.4; $MgSO_4$, 1.2; $CaCl_2$, 1.3; $NaHCO_3$, 25, adjusted to pH 7.4.

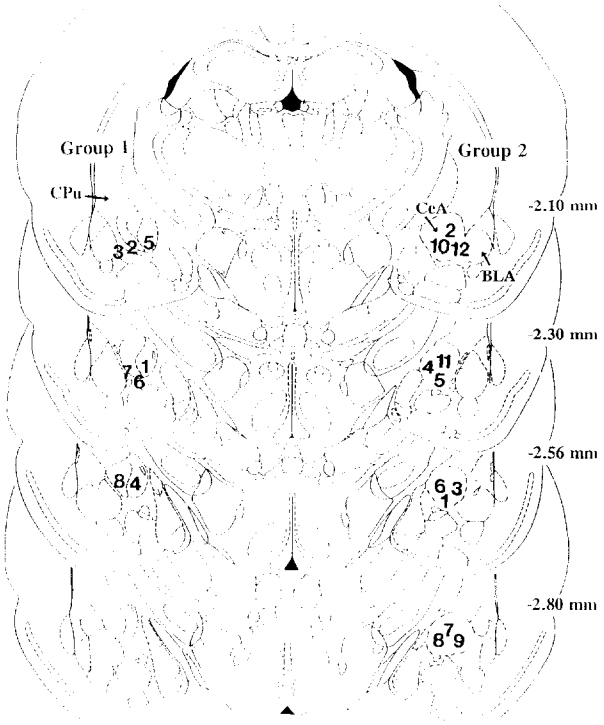
RESULTS

The cocaine (10 mg/kg) vs. saline discrimination was acquired in an average of 22 sessions (range: 10–35). Throughout acquisition, response rates (±SEM) during cocaine sessions (58.3 ± 8 responses/min) were not significantly different from those observed during saline sessions (49.5 ± 11 responses/min).

Administration of systemic cocaine (0.625–20 mg/kg) produced a dose-dependent increase in drug-appropriate responding (Fig. 2, right) whereas saline administration engendered <10% drug-lever responses. The dose of cocaine predicted to produce 50% cocaine-lever responding (ED_{50}) was 1.6 mg/kg. Response rates were stable across all test doses of systemic cocaine and were not significantly different from the previous cocaine session. Performance (cocaine-lever responding and response rates) following cannulation was not significantly different from that observed prior to cannulae implants (data not shown).

Intracranial injection of cocaine (40–200 μg) into the central amygdala produced, at best, a partial substitution (<60% drug-lever responding; Fig. 2, left). Microinjection of either ACSF (1 μl/side; Fig. 2) or the DA D_1 receptor antagonist SCH 23390 (0.5–2 μg; Fig. 3) resulted in primarily saline-appropriate responding (<20% drug-lever responding). Response rates following intracranial administration of either ACSF, cocaine, or SCH 23390 were significantly lower than those from the previous cocaine session.

In combination (antagonism) tests, administration of SCH 23390 (0.5–2 μg) into the central amygdala resulted in a dose-related antagonism of a dose of cocaine (5 mg/kg, IP) that engendered >85% cocaine-lever responses when given alone (Fig. 4). There was a significant effect of SCH 23390 dose upon the percentage of drug-lever responding engendered by cocaine (5 mg/kg), $F(3, 16) = 3.14$, $p = 0.05$. The dose of SCH 23390 predicted to antagonize cocaine-lever responding by 50% (AD_{50}) was 0.66 μg. Response rates following administration of intra-amygdala SCH 23390 (0.5–2 μg) in combination with cocaine (5 mg/kg) were not significantly different from those observed following this dose of cocaine alone, $F(3, 16) = 0.80$, $p = 0.51$. In addition, intra-amygdala microinjection of a fixed dose of SCH 23390 (2 μg) with various doses of cocaine (2.5–20 mg/kg, IP) resulted in a rightward shift in the cocaine dose-response curve (Fig. 5). Intra-amygdala infusion of SCH 23390 (2 μg) produced a significant effect upon the cocaine dose-response curve, $F(3, 30) = 4.64$, $p = 0.009$. The ED_{50} for cocaine following pretreatment with intra-amygdala SCH 23390 was 6.6 mg/kg compared to the ED_{50} (1.5 mg/kg) observed for cocaine prior to SCH 23390 administration. Response rates following administration of intra-amygdala SCH 23390 in combination with various doses of



(A)

FIG. 1. Histological assessment of cannulae implants in the amygdala. In the top panel, coronal sections indicate placement of the cannulae tips in the central amygdala according the atlas of Paxinos and Watson (31); reference to distance in millimeters posterior to bregma is provided on the right side of the figure. Each identification number for animals in group 1 (N = 8; left side) and group 2 (N = 12; right side) represents that subject's bilateral cannulae placement within the amygdala. In the bottom panel, a coronal brain section representing placement of the bilateral implants located within the central amygdala (anterior/posterior plane is −2.56 mm from bregma). Anatomical abbreviations: basolateral amygdala (BLA), central amygdala (CeA), and caudate putamen (CPu).

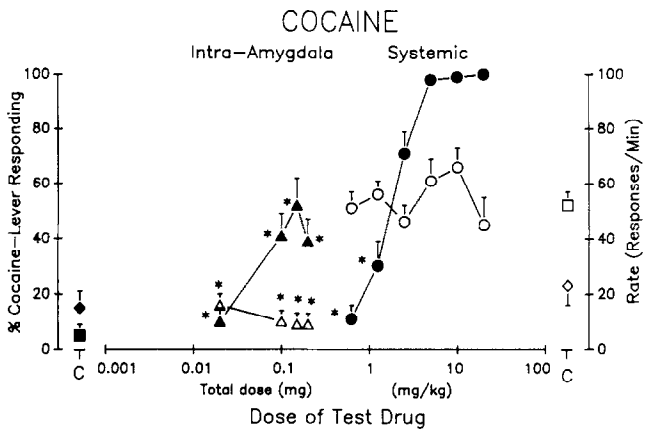


FIG. 2. Results of intra-amygdala and systemic injections of cocaine in animals trained to discriminate cocaine (10 mg/kg) from saline. Closed symbols denote the mean percentage of cocaine-appropriate responses (±SEM; left ordinate); open symbols denote the mean number of responses/min (±SEM; right ordinate). Triangles and circles represent intra-amygdala and systemic (IP) injections of cocaine, respectively. For comparison, the percentage of cocaine-lever responding and response rate observed following saline (squares) and ACSF (diamonds) control tests are included (C). All systemic cocaine data points represent the means of data from 20/20 rats [the number of rats (n) completing the FR 20 on either lever out of the number of rats tested (N)] with the exception of cocaine (20 mg/kg), which is the means of data from 6/6 rats. Intra-amygdala cocaine data points represent the means of data from 8/8 rats. Asterisks represent performances during test sessions that were significantly different from the previous cocaine training session ($p < 0.05$).

cocaine were significantly different from those obtained with cocaine alone, $F(3, 30) = 3.94, p = 0.01$.

DISCUSSION

The primary aim of the present study was to investigate the role of the amygdala and, in particular, central amygdala DA

FIG. 3. Results of intra-amygdala administration of the DA D_1 antagonist SCH 23390 in animals trained to discriminate cocaine (10 mg/kg) from saline. See Fig. 2 for explanation of symbols. For comparison, percentage of cocaine-lever responding and response rates observed following systemic injection of saline (squares) and intra-amygdala ACSF (diamonds) are shown. All data points represent the means of data from 6/6 rats. Asterisks represent performances during test sessions that were significantly different from the previous cocaine session ($p < 0.05$).

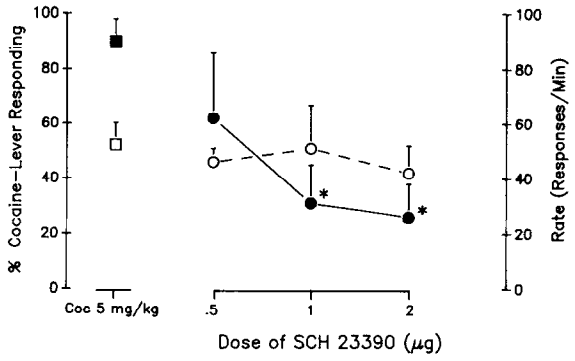


FIG. 4. Results of intra-amygdala administration of the DA D_1 antagonist SCH 23390 in combination with a single dose (5 mg/kg) of cocaine. See Fig. 2 for explanation of symbols. For comparison, percentage of cocaine-lever responding and response rate observed after systemic injection of cocaine alone is shown (Coc 5 mg/kg; squares). All data points represent the means of data from 5-6/6 rats. Asterisks represent performances during test sessions that were significantly different from cocaine (5 mg/kg) alone ($p < 0.05$).

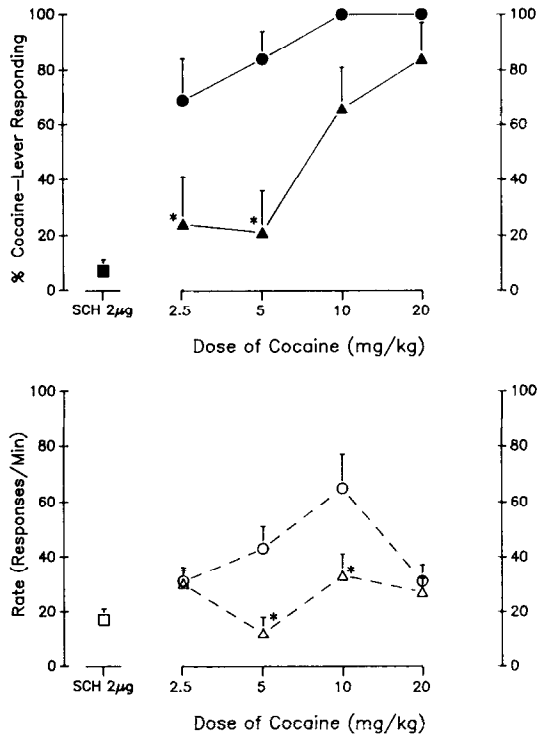


FIG. 5. Results of intra-amygdala administration of the DA D_1 antagonist SCH 23390 in combination with various doses (2.5-20 mg/kg) of cocaine. See Fig. 2 for explanation of symbols. Triangles represent intra-amygdala SCH 23390 (2 μg) in combination with various doses of cocaine. For comparison, percentage of cocaine-lever responding and response rates observed following doses of cocaine alone (2.5-20 mg/kg; circles) and intra-amygdala injection of SCH 23390 alone are shown (SCH 2 μg; squares). All data points represent the means of data from 6/6 rats. Asterisks represent performances during test sessions that were significantly different from the particular dose of cocaine (2.5-20 mg/kg) administered alone ($p < 0.05$).

D_1 receptors in modulating the discriminative stimulus effects of cocaine. Whereas intracranial mircoinjections of cocaine into the central amygdala did not completely substitute for the systemic cocaine cue, intra-amygdala infusions of the DA D_1 receptor antagonist SCH 23390 produced a dose-dependent and complete blockade of a systemic dose of cocaine (5 mg/kg). Furthermore, infusion of a fixed dose of SCH 23390 (2 μg) into the central amygdala resulted in a rightward shift in the cocaine dose-response curve. Although an intra-amygdala vehicle infusion was not tested for antagonism of the cocaine cue, several findings argue against the possibility that a nonspecific disruption of discriminative behavior was induced by intra-amygdala SCH 23390. First, if vehicle microinjections disrupted discriminative performance, drug-lever responding might be expected following microinjections of ACSF; however, this was not the case. Second, intra-amygdala injections of SCH 23390 (2 μg) in combination with various doses of cocaine (Fig. 5) resulted in a relatively parallel, rightward shift in the cocaine dose-effect curve. If infusion of SCH 23390 produced nonspecific effects, a flattening of the cocaine dose-response curve would be expected following all intracranial injections. Lastly, the ability of increasing doses of intra-amygdala SCH 23390 (0.5–2 μg) to dose-dependently antagonize the systemic cocaine response (Fig. 4) suggests further that a specific, rather than nonspecific, pharmacological effect occurred following intracranial infusions of the test drugs into the amygdala. Therefore, the results of the present investigation suggest that DA D_1 receptors localized within the central amygdala are important in mediating the discriminative stimulus properties of cocaine.

The specificity of the intra-amygdala drug infusion is supported by the precise placements of the implant cannulae observed upon postmortem histological examination. However, there is undeniably spread of drug from the injection site (43) and diffusion of drug to adjacent brain structures could contribute to the present results. The caudate-putamen, which lies directly above the amygdala, is the most likely brain area to be affected due to efflux of infused drug up the cannulae tract (43). However, the inability of intracaudate cocaine to fully substitute for the cocaine cue (44) or for intracaudate SCH 23390 to block cocaine self-administration (4,27) suggests that diffusion of test drugs from the amygdala to the adjacent caudate is probably not the basis for our current results. Furthermore, Caine and colleagues (4) have recently demonstrated the diffusion rate of [^{3}H]SCH 23390 injected into the amygdala; these data suggest that, at the time intervals used in the present study (20 and 45 min), SCH 23390 remains largely localized within the amygdala.

The nucleus accumbens appears to be a primary mesolimbic brain region involved in mediating the discriminative stimulus effects of psychostimulants (7,16,17,30,44). For example, administration of cocaine (7,44) directly into the nucleus accumbens fully substitutes whereas intra-accumbens microinjection of a DA D_1 receptor antagonist blocks the systemic cocaine cue (7). Thus, the relative roles of the nucleus accumbens and amygdala are not identical as administration of cocaine into the amygdala failed to mimic the cocaine cue, although intra-amygdala infusions of SCH 23390 effectively antagonized the discriminative stimulus effects of cocaine. These findings actually parallel reports concerning the relative participation of these two mesolimbic regions in the generation of DA-mediated motor behavior (12,13,32). For example, Costall and colleagues (12,13) demonstrated that microinjection of DA, apomorphine or (−)-*N*,*n*-propylnorapomorphine directly into the nucleus accumbens resulted in stereotyped sniffing and hyperactivity in a manner similar to intra-accumbens amphetamine (32), whereas microinjections of these DA compounds into the central amygdala failed to alter motor behavior. Electrolytic lesions of the central amygdala, however, were reported to block both amphetamine- and apomorphine-induced stereotypies (10,11) whereas administration of the DA D_1 receptor antagonist SCH 23390 into the amygdala blocked cocaine self-administration (4,28) and cocaine-induced hyperlocomotion (28). Thus, neither DA-mediated motor behavior nor the discriminative stimulus effects of cocaine are readily generated via DA stimulation within the amygdala yet ablation of the amygdala or blockade of amygdala DA receptors does attenuate the behavioral response to systemic DA agonists. Therefore, the ability of intra-amygdala administration of the DA D_1 receptor antagonist SCH 23390 to dose-dependently antagonize the discriminative stimulus effects of cocaine suggests that the amygdala and, in particular, amygdala DA D_1 receptors (40) are critically involved in modulation of this behavioral effect.

Nucleus accumbens DA D_1 and D_2 receptors are involved in modulating the reinforcing (27,28,38), discriminative stimulus (7,30), and hyperlocomotor effects of psychostimulants (14,28). However, a functional dissociation has been observed between the accumbens and the amygdala regarding the manner in which DA D_1 receptors in these nuclei modulate cocaine reinforcement. McGregor and Roberts (28) demonstrated that the *rate* of cocaine intake increased to a greater extent following intra-amygdala, as opposed to intra-accumbens, SCH 23390 infusion. On the other hand, intra-accumbens SCH 23390 was more effective at decreasing the *breakpoint* on a progressive ratio schedule of cocaine self-administration. These two indices (rate of intake vs. breakpoint) may measure aspects of cocaine reinforcement that are differentially mediated by DA D_1 receptors in the amygdala and accumbens, respectively. These authors suggest that the maintenance of cocaine self-administration may be related to its interoceptive (subjective) properties whereas breakpoint performance under a progressive ratio schedule is related to the level of motivation to acquire cocaine (i.e., reinforcing efficacy). Thus, blockade of the stimulus ("subjective"; present results) effects of cocaine with intra-amygdala SCH 23390 may account for the observed reduction in its reinforcing effects (4,28) or perhaps the recognition of cocaine as an interoceptive stimulus is disrupted due to an interruption in the stimulus-reward and/or learning and memory functions for which the amygdala is responsible (1,29,34). However, the amygdala is not the primary limbic region responsible for mediating the "subjective" effects of cocaine because intra-accumbens (7,44), but not intra-amygdala (present results), cocaine completely mimics the systemic cocaine cue. Furthermore, infusion of SCH 23390 into the nucleus accumbens proved to be a more potent cocaine antagonist (7) compared to intra-amygdala SCH 23390 (present results); that is, the dose of intra-accumbens SCH 23390 predicted to antagonize the cocaine response by 50% (AD_{50}) was 0.09 μg compared to the AD50 for intra-amygdala SCH 23390 which was 0.66 μg. Therefore, these results would tend to suggest that the nucleus accumbens plays a greater role in mediating the discriminative stimulus (subjective?) effects of cocaine whereas, the reinforcing effects of cocaine may be differentially mediated by the two limbic regions depending upon the schedule of reinforcement (fixed vs. progressive ratio) that is used.

Several amygdala nuclei, including the central, lateral, and basolateral nuclei, innervate the nucleus accumbens (2,23,46) and, although the neurochemical identity of this amygdala-

accumbens pathway has not been fully characterized, aspartate and/or glutamate fibers appear to participate (9,36). Electrophysiological studies have demonstrated that electrical stimulation of the central and basolateral amygdala can activate nucleus accumbens neurons (8,24,45). Moreover, the basolateral amygdala-evoked excitatory response in the accumbens was reduced substantially by iontophoretic application of either DA D_1 or D_2 agonists as well as stimulation of the ventral tegmental nucleus (24,45). Central and basolateral amygdala neurons also respond to DA in an inhibitory fashion (3,6) and, although the receptor subtype that mediates this inhibition is uncharacterized, DA D_1 and D_2 receptors are found in both amygdala subnuclei (40). Thus, blockade of amygdala DA D_1 receptors might interrupt the excitatory amygdala pathway to the nucleus accumbens, resulting in a dampening of the discriminative stimulus effects of cocaine that are critically dependent upon accumbens mechanisms (7,17,44). In addition, the amygdala innervates dopaminergic cells of the midbrain (2,42,46) in a physiologically relevant manner (26,29) and blockade of amygdala DA D_1 receptors could also alter the functional integration of these midbrain DA projections to the nucleus accumbens. Based upon these findings, one might predict that intra-amygdala microinjection of SCH 23390 would result in an antagonism of the discriminative stimulus effects of intra-accumbens cocaine. This hypothesis should be tested separately for subnuclei of the amygdala (e.g., central vs. basolateral) because the relative density of accumbens innervation (2,19,23) and the proportion of accumbens neurons that respond electrophysiologically to amygdala stimulation differs (26,45). As such, the relative role of specific amygdala subnuclei may differ substantially.

In summary, blockade of DA D_1 receptors localized within the amygdala (present results) or nucleus accumbens (7) results in a dose-dependent and complete antagonism of the discriminative stimulus properties of cocaine. Although blockade of accumbens D_1 receptors may act directly to reduce the "subjective" effects of cocaine, blockade of DA D_1 receptors located within the amygdala may reduce the cocaine stimulus as a consequence of interrupting the functional pathway between the amygdala and the nucleus accumbens.

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