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Effects of the D_1-Antagonist SCH23390 and the D_2-Antagonist Eticopride on Self-Administration of the D1-Agonist SKF82958

Key Terms:

- Dopamine
- Agonist
- Antagonist
- SCH23390: D_1-Antagonist
- Eticlopride: D_2-Antagonist
- SKF82958: D_1-Agonist

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Mohammad Helmy recalls that his favorite research experiences were when he and his faculty mentor performed statistical analyses of the data and brainstormed about an explanation for the results. Mohammad says that research affected him to such an extent that he no longer accepts scientific "facts" without first questioning the metho-dology used in accumu-lating the data. Mohammad recommends that undergraduate researchers realize at all times the contributions they are making to the community around them.

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Abstract

Cocaine is believed to exert its reinforcing actions by indirectly increasing activation of postsynaptic dopamine (DA) receptors (divided into five subtypes, D_1 to D_5). We studied the role of the D_1 receptor in reinforcement by examining the effects of the D_1-antagonist SCH23390 and the D_2-antagonist eticlopride on self-administration of the D_1-agonist SKF82958. Rats were surgically implanted with an intravenous catheter, then allowed to self-administer cocaine during daily sessions until response rates stabilized. On subsequent test days, rats were allowed to self-administer SKF82958 alone or in combination with either SCH23390 or eticlopride. As the SCH23390 dose was increased, self-administration of the low dose of SKF82958 decreased; responses for the higher SKF82958 doses initially increased before eventually declining. Eticlopride caused a decrease in self-administration of the lower SKF82958 doses, but caused no change in self-administration of the higher doses. SCH23390 disrupted the highly regular response patterns that are indicative of reinforcement, while eticlopride had no effect on these response patterns.

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Faculty Mentor

The rewarding effects of cocaine have been traced mainly to its enhancement of brain dopamine (DA) systems. With five DA receptor subtypes (D_1 to D_5), it is important to determine which subtype is affected by cocaine. This study replicated the finding that self-administration of D_1-agonists produces well-timed, cocaine-like response patterns, and showed that D_1-agonist self-administration is antagonized by blockade of D_1 receptors, but largely unaffected by D_2 blockade. These results support a role for D_1 receptors in reinforcement, and suggest that cocaine's actions involve stimulation of dopamine D_1

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receptors—a fact that might help elucidate the mechanism of action of this important drug. More generally, UCI's undergraduate research program allows students to experience laboratory research in pursuit of scientific answers to real research questions.

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Introduction

Cocaine is a stimulant with powerful reinforcing properties that strengthen drug-seeking behavior and make it difficult for addicts to abandon its use. Cocaine is thought to achieve its reinforcing actions by inhibiting the presynaptic reuptake of dopamine (DA), resulting in the accumulation of DA in the synaptic cleft.[1] The elevated levels of synaptic DA enhance activation of postsynaptic DA receptors, which have been divided into five subtypes, D_1 to D_5.[2,3,4,5] Research has focused on the involvement of D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors in mediating reinforcement, since past studies have demonstrated that self-administration of D_1 and D_2 agonist combinations mimic cocaine self-administration patterns in rats.[6]

Previous findings also suggest that while D_2-mediated reinforcement may require some threshold level of endogenous D_1 activation, D_1-mediated reinforcement does not depend on D_2 activity.[7,8] To further study the properties of D_1-mediated reinforcement, the present study examines the effects of the D_1-selective antagonist SCH23390 and the D_2-selective antagonist eticlopride on self-administration of the D_1-selective agonist SKF82958. Since D_1 and D_2 antagonists have been found to cause compensatory increases in cocaine self-administration and to shift the cocaine dose-response curve to the right, we expect SCH23390 to have the same effect on SKF82958 self-administration.[9] If past findings by Self et al. are correct, and if D_1-mediated reinforcement does not depend on D_2 activation, we expect eticlopride to have no specific effect on SKF82958 self-administration.[8]

In a similar study by Self et al., the antagonists were administered subcutaneously 30 min before each experimental session, while the agonist was self-administered intravenously during the session.[8] This methodology is disadvantageous because it results in an inconsistent, time-dependent antagonist dose, where the effective antagonist dose rises and peaks early in the session, then declines as the session progresses. To avoid this inconsistent dosing, our study co-administers the antagonist with each self-administered agonist injection, such that both drugs are simultaneously and rapidly distributed throughout the circulation. Our

Materials and Methods

N□ive, male Sprague-Dawley rats, initially weighing 260 to 300 g, were maintained under a 12 h light/dark cycle (lights on at 7:00 a.m., experiments conducted during light phase). Animals were individually housed, handled daily, and provided with food and water ad libitum. After 3 to 4 d of habituation, rats were food-deprived in preparation for individual lever-press training. Training was conducted in an experimental chamber (27 cm x 25 cm x 30 cm) equipped with a lever mounted on the back wall, a white cue light situated above the lever, and a food bin adjacent to the lever. During the daily 20 min training sessions, each rat received one 45 mg food pellet in the food bin for each lever-press (10 g minimum force). Training continued until the rat completed three consecutive days of 100 lever-presses per session.

Trained rats were then surgically implanted with an autoclaved chronically indwelling jugular catheter. Catheters consisted of a 12 cm length of Silastic7 tubing connected to a guide cannula (22-gauge) bent into a 95□ angle and encased in a hemispherical cranioplastic cement base. A 3.5 cm square of Marlex7 mesh was cemented to the bottom of the base, and a 1.0 cm square of Mersilene7 mesh was glued (Medical Adhesive Silicone Type A) onto the tubing 3.25 cm from its end. Rats were anaesthetized with an intraperitoneal injection of equithesin (0.35 ml/100 g body weight). The base of the catheter was placed in a 4 cm incision pocket on the back of the rat beneath the dermal layer. The tubing was passed subcutaneously over the shoulder and inserted into the external jugular vein, to which the 1 cm square mesh was sutured (4-0 silk thread). The incisions were sutured, and the portion of the guide cannula emerging from the animal's back was capped with a stylet to prevent infection and clogging. Implanted catheters were flushed twice daily with 0.2 to 0.3 ml of a heparin-saline solution (0.6 ml heparin/30 ml bacteriostatic 0.9% NaCl solution).

Animals began self-administration sessions following a 3 d recovery period and were maintained at weights between 350 to 400 g. The daily 3 h self-administration sessions were conducted in individual test chambers identical to the one used for lever-press training, however now the food bin was concealed. At the start of each session, the rat's guide cannula was

study also differs from previous ones in that we tested more doses of SKF82958 and used the potent and selective D_2 antagonist, eticlopride. By making these modifications we hope to replicate and augment precision and thoroughness in our continued investigation of D_1-mediated reinforcement.

connected to an external syringe pump system by plastic tubing. A single press of the lever activated the syringe pump to deliver a 0.1 ml injection of drug solution, lasting 6 s and accompanied by an audible 1800 Hz tone. The injection period was followed by a 10 s "time out" period during which the cue light was turned off, and the

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lever presses were not reinforced. At the start of each session, rats received two priming injections to signal the availability of drug. The syringe pump system, cue light, and tone were controlled by an IBM PC computer via a LabLinc I/O panel. The computer recorded both reinforced and non-reinforced lever presses.

Rats were allowed to self-administer cocaine (750 µg/kg/inj) until their response rates stabilized (±10% for two consecutive days). On subsequent test days, rats were allowed to self-administer SKF82958 (3, 10, or 30 µg/kg/inj) alone or in combination with either SCH23390 (0.1, 0.3, 1, 3, or 10 µg/kg/inj) or eticlopride (0.3, 1, 3, or 10 µg/kg/inj). Drug combinations and doses were randomly assigned. All drugs were dissolved in bacteriostatic 0.9% NaCl solution, and drug solutions were sterile filtered (0.2 µm Acrodisc). The volume of saline vehicle added was calculated based on the rat's weight, measured 45 min before each session. Cocaine was provided by the National Institute of Drug Abuse. SKF82958 [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], SCH23390 [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl- 2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride], and eticlopride [S(-)-3-chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride] were obtained from Research Biochemicals Incorporated.

Statistical computations were performed using Systat 6.0. Response rates at each dose of SKF82958, alone versus in combination with each antagonist, were analyzed using a one-way ANOVA and posthoc tests with Bonferroni adjustments.

Results

Figure 1 shows the effects of SCH23390 dose on self-administration of each dose of SKF82958. When combined with the low dose of SKF82958 (3 µg/kg/inj), the lowest dose of SCH23390 (0.1 µg/kg/inj) caused a small increase in response rate. Further increases in the SCH23390 dose (0.3, 1, 3 µg/kg/inj) produced significant decreases in self-administration of SKF82958 3 µg/kg/inj. SCH23390 exerted similar, but more pronounced, effects on self-administration of the higher SKF82958 doses (10 and 30 µg/kg/inj). Responses for SKF82958 10 µg/kg/inj initially increased with increasing SCH23390 dose (0.1 and 0.3 µg/kg/inj) before rapidly declining at the higher

Similarly, responses for SKF82958 30 µg/kg/inj increased significantly with increasing antagonist doses and declined at SCH23390 3 µg/kg/inj.

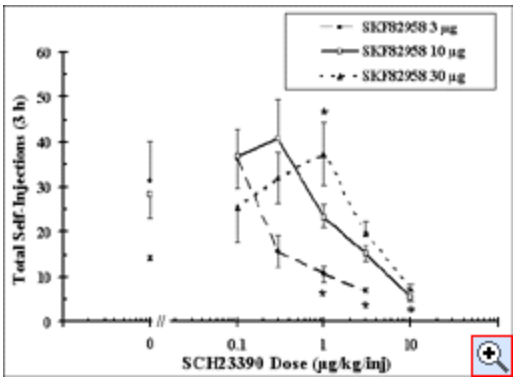

Figure 1
Effect of SCH23390 (0.1, 0.3, 1, 3, 10 ug/kg/inj) on self-administration of SKF82958 (3, 10, 30 ug/kg/inj). Data are presented as mean total self-injections during 3 hr sessions. *p<0.05 versus same dose of SKF82958 alone.

The effect of eticlopride dose on self-administration of each dose of SKF82958 is shown in Figure 2. Self-administration of SKF82958 3 µg/kg/inj decreased with increasing eticlopride dose to significantly lower levels than baseline. When combined with the intermediate SKF82958 dose (10 µg/kg/inj), lower doses of eticlopride (0.3 and 1 µg/kg/inj) did not affect response rate. Self-administration began to decrease at 3 µg/kg/inj of eticlopride, and the decline paralleled that seen in self-administration of 3 µg/kg/inj SKF82958 in combination with similar eticlopride doses. Self-administration of 30 µg/kg/inj SKF82958 was unaffected by the lower eticlopride doses (0.3 and 1 µg/kg/inj). Responses were decreased by eticlopride 3 µg/kg/inj and remained unchanged when the antagonist dose was increased further. However, unlike the fall in response rates for

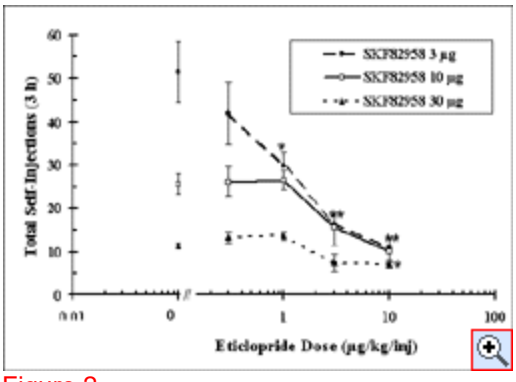

Figure 2
Effect of eticlopride (0.3, 1, 3, 10 ug/kg/inj).

antagonist doses, starting at 1 g/kg/inj.

Data are presented as mean total self-injection during 3 h sessions. *p<0.05 versus same dose of SKF82958 alone, **p<0.01 versus same dose of SKF82958 alone.

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SKF82958 3 and 10 μg/kg/inj, the decline in responses for SKF82958 30 μg/kg/inj was not statistically significant.

Self-administration of SKF82958 30 μg/kg/inj alone produced highly regular response patterns and evenly spaced inter-response intervals (Figures 3 and 4). Low doses of SCH23390 (0.1 and 0.3 μg/kg/inj) decreased the inter-response intervals for SKF82958 30 μg/kg/inj self-administration, while maintaining the regular overall response patterns (Figure 3). However, when combined with higher doses of antagonist (1, 3, 10 μg/kg/inj), responses became erratic and the regular self-administration pattern was lost. With the exception of the 3 μg/kg/inj dose, eticlopride did not significantly affect the regularity of response patterns for 30 μg/kg/inj SKF82958 (Figure 4).

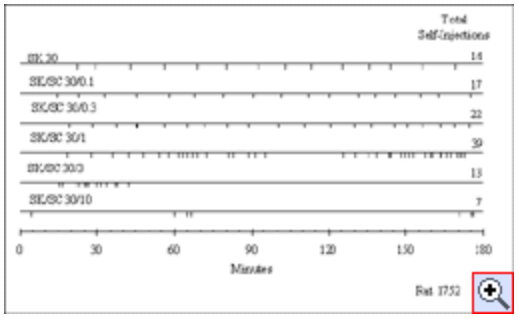

Figure 3
Response pattern of a representative rat self-administration SKF82958 30 mg/kg/inj alone and in combination with SCH23390 (0.1, 0.3, 1, 3, 10 mg/kg/inj). Each vertical tick mark represents one self-injection. Total self-injections per 3 h session are shown at the end of each line. (SK=SKF82958, SC=SCH23390)

Discussion

Our study examined the effects of SCH23390 and eticlopride on SKF82958 self-administration. The effects of SCH23390 on response rate and pattern of SKF82958 self-administration suggest that the two drugs are competing for the same binding site (D_1 receptor), and that blocking the D_1 receptor attenuates reinforcement mediated by SKF82958. As shown in Figure 1, low doses of SCH23390 reduced self-administration of the low dose of SKF82958 (3 μg/kg/inj), but caused an increase in response rate for the higher SKF82958 doses (10 and 30 μg/kg/inj). Rats increased self-administration in the latter case in an effort to compensate for the effects of the antagonist, an important indication that the two drugs are competing for the same site, and that the antagonism SCH23390 dose-response curve (Figure 1). Increasing the agonist dose caused a rightward shift in the antagonist dose-response curve, another indication of competitive pharmacological antagonism that could be surmounted by increasing the agonist dose.

Figure 3 presents further evidence of competitive and surmountable antagonism by SCH23390. Although low doses of SCH23390 (0.1 and 0.3 μg/kg/inj) caused compensatory increases in SKF82958 30 μg/kg/inj self-administration, they did not completely block reinforcement since rats maintained highly regular response patterns. Higher doses of antagonist, however, produced erratic patterns of response indicative of attenuated reinforcement that could not be overcome by increasing self-administration. As seen in Figure 3 for SCH23390 3 μg/kg/inj, the rat was unable to surmount the antagonist's effect despite rapid successive self-injections, resulting in diminished reinforcement and extinction of self-administration.

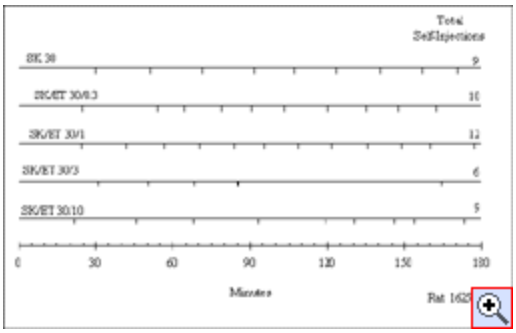

Figure 4
Response pattern of a representative rat self-administration SKF82958 30 ug/kg/inj alone and in combination with eticlopride (0.3, 1, 3, 10 ug/kg/inj). Each vertical tick mark represents one self-injection. Total self-injections per 3 h session are shown at the end of each line. (SK=SKF82958, ET=Eticlopride)

In contrast to SCH23390, the effects of eticlopride on the rate and pattern of SKF82958 self-administration suggest that the two drugs are not competing for the same binding site, and that blockade of the D_2 receptor by eticlopride does not attenuate reinforcement mediated by SKF82958 at the D_1 receptor. As shown in Figure 4, with the exception of the 3 μg/kg/inj antagonist dose, eticlopride had little or no effect on the pattern of SKF82958 30 μg/kg/inj self-administration. This suggests that, unlike SCH23390 (Figure 3, lines 1 and 6), eticlopride did not block D_1 reinforcement mediated by SKF82958, indicated by the fact that the highest dose of

could be overcome by increasing agonist intake. This point is further demonstrated in the effect of SKF82958 on the eticlopride yielded a pattern of self-administration similar to the one produced by self-administration of SKF82958 30 μg/kg/inj alone (Figure 4, lines 1 and 5).

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Although blockade of the D_2 receptor by eticlopride had little effect on SKF82958 response patterns, it did cause a insurmountable dose-dependent decrease in SKF82958 self-administration (Figure 2). One explanation is that eticlopride is non-selectively binding and antagonizing the D_1 receptor. This is unlikely since we do not observe a compensatory increase in response rate that is indicative of competitive pharmacological antagonism, as seen with SCH23390 (Figure 1).

Another possible explanation of the suppressive effects of eticlopride on response rate is that blocking D_2 receptors attenuates D_1 reinforcement. Though it has been suggested that D_2 reinforcement may depend on D_1 activation, the reciprocal scenario in which D_1 reinforcement requires D_2 activation has not been found to be true.[7,8,10] Furthermore, if D_1 reinforcement did require D_2 activation, then blockade of the D_2 receptor by eticlopride should dose-dependently decrease self-administration of all doses of SKF82958 to the same extent, since SKF82958 acts primarily at the D_1 receptor. Figure 2, however, shows that attenuation of responses is dependent on the dose of SKF82958, with higher doses of eticlopride required to decrease self-administration of higher doses of SKF82958.

Previous studies have shown that eticlopride, like other DA antagonists, may decrease behavior and motor activity.[11,12] The decreases in response rates caused by eticlopride may be attributed to its non-specific antagonism of motor activity and not to pharmacological antagonism of D_1 reinforcement.

As the dose of eticlopride increases, the duration of its effects increases and may outlast the time between successive self-injections. At the SKF82958 dose with a higher baseline number of responses (3 μg/kg/inj), the suppressive effects on motor activity may outlast the baseline inter-response interval and impede the rat's ability attempt for the next self-injection. As a result, the rat must wait longer between successive responses and self-administers less drug during the 3 h session (Figure 2). In contrast, at the SKF82958 dose with a lower baseline (30 μg/kg/inj), the suppressive effects of eticlopride dissipate during the longer baseline inter-response interval. As a result, the time between successive self-injections is unaffected, and the total number that they are likely due to suppression of motor activity, not to pharmacological attenuation of D_1-mediated reinforcement.

In contrast to findings that D_2-mediated reinforcement may depend on some threshold level of D_1 activation, our results suggest that D_1-mediated reinforcement does not depend on D_2 activation, and that D_1 reinforcement by SKF82958 can be pharmacologically antagonized by the D_1 antagonist SCH23390, not the D_2 antagonist eticlopride.

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of responses during the 3 h session remains relatively constant (Figure 4).

Furthermore, the dose-response curves for 3 and 10 g/kg/inj SKF82958 overlap at the higher eticlopride doses (1, 3, 10 g/kg/inj), suggesting that the suppression of motor activity may limit the number of self-injections attainable by the rat in the 3 h session, independent of the dose of SKF82958 (Figure 2). Further evidence of this is seen with the 10 g/kg/inj eticlopride dose, at which response rates for all doses of SKF82958 are almost identical. These results strengthen the idea that the effects of eticlopride on SKF82958 self-administration are non-specific, and

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