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The selective dopamine uptake inhibitor, D-84, suppresses cocaine self-administration, but does not occasion cocaine-like levels of generalization

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Abstract

A successful replacement pharmacotherapy for treating cocaine dependency would likely reduce cocaine's abuse, support a low abuse liability, overlap cocaine's subjective effects, and have a long duration of action. Inhibitors with varying selectivity at the dopamine transporter (DAT) have approximated these properties. The objective of the present study was to characterize the behavioural effects of an extremely selective DAT inhibitor, (+) trans-4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl) piperadin-3-ol (D-84), a 3-hydroxy substituted piperidine derivative of GBR-12935, for its cocaine-like discriminative stimulus effects, its effects on cocaine self-administration, and for its own self-administration. During cocaine discrimination tests, cocaine occasioned the 10 mg/kg cocaine training stimulus with an ED_{50} value of 3.13 (1.54-6.34) mg/kg, and reduced response rates with an ED_{50} value of 20.39 (7.24-57.44) mg/kg. D-84 incompletely generalized to the cocaine stimulus occasioning a maximal 76% cocaine lever responding, while reducing response rates with lower potency than cocaine (ED_{50}=30.94 (12.34-77.60) mg/kg). Pretreatment with D-84 (9.6-30.4 mg/kg) significantly ($P<0.05$) reduced cocaine intake at 17.1 mg/kg D-84 when cocaine was self-administered at 0.5 mg/kg/infusion, and at 30.4 mg/kg D-84 when cocaine was self-administered at 0.1, 0.5 .and 1.0 mg/kg/infusion. During self-administration tests with D-84 (0.1-1 mg/kg/infusion), numbers of infusions significantly exceeded vehicle levels at 0.3 mg/kg/infusion. These results show that D-84 pre-treatment can decrease cocaine intake especially when high doses of cocaine are being self-administered. This observation, combined with its incomplete generalization to the cocaine discriminative stimulus and its reported long duration of action, provides a profile consistent with a potential replacement therapy for treating cocaine abusing patients.

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dopamine uptake inhibitor; cocaine; self-administration; drug discrimination; drug abuse treatment

1. Introduction

Cocaine abuse and addiction is a persistent, worldwide, socioeconomic and medical problem and an estimated 1.5 million people are addicted to cocaine (SAMHSA, 2000). Studies have shown that cocaine inhibits the uptake of serotonin, dopamine and noradrenaline (Reith et al., 1986; Taylor and Ho, 1978), although the preponderance of its abuse-related effects are likely attributable to its inhibition of the dopamine transporter (DAT) (Ritz et al., 1987).

It has been hypothesized that a selective DAT inhibitor may provide an effective therapy for cocaine dependent individuals, and to this end DAT inhibitors have been investigated as potential replacement therapies for cocaine abuse (Bergman et al., 1989; Carroll et al., 2006; Spealman et al., 1989). Tropane analogues were among the first DAT inhibitor compounds that were investigated as cocaine replacement therapies. [3β-(4-chlorophenyl)tropane-2β-carboxylic acid phenyl ester hydrochloride (RTI-113) and 2beta-propanoyl-3beta-(4-tolyl)-tropane (PTT) both have a high selectivity for DAT versus the serotonin (SERT) and noradrenaline (NET) transporters, and both have been shown to decrease cocaine self-administration in non-human primates; however, both compounds also have undesirable side effects at doses similar to those needed to decrease cocaine self-administration (Birmingham et al., 1998; Negus et al., 2009). The effects of benztropine analogues have also been investigated and have been shown to bind to DAT with a relatively high affinity, are less effective than cocaine in producing positive reinforcing effects, and have a longer duration of action, all of which are properties thought desirable for a cocaine replacement therapy (Hiranita et al., 2009; Katz et al., 2001). The dialkenyl-piperazine derivative, GBR 12909, has also been investigated as a potential cocaine replacement therapy. GBR 12909 has moderate DAT selectivity (Van der Zee et al., 1980), with discriminative stimulus effects similar to cocaine (Koetzner et al., 1996), and has been reported to reduce cocaine self-administration while being self-administered by rodents and non-human primates (Wojnicki and Glowa, 1996).

RTI-113, PTT, benztropin analogues and GBR 12909 possess several effects thought propitious for a cocaine replacement pharmacotherapeutic, including an ability to occasion the cocaine discriminative stimulus and to reduce cocaine self-administration, while having a relatively long duration of activity; however, none have yet become approved as a pharmacotherapy for cocaine-dependence (Birmingham et al., 1998; Nader et al., 1997; Walsh et al., 2000; Wojnicki and Glowa, 1996). It is unknown whether a drug with even greater selectivity for the DAT would have an improved profile. ((+) trans-4-(2-Benzhydryloxyethyl)-1-(4-fluorobenzyl) piperadin-3-ol) (D-84) is a 3-hydroxy substituted piperidine derivative of GBR-12935, and is, to date, one of the most potent (IC50=0.46 nM ±0.05) and selective ligands for the DAT relative to the SERT and NET amine transporters with over a 7000 and 4000 fold greater selectivity, respectively (Ghorai et al., 2003). D-84 is also selective for the DAT versus other receptors. In binding assays involving 63 receptors, D-84 only had affinity with a potency of ≤1000 nM for the dopamine D4 (28.9±24.2 nM), histamine H2 (909.5±57 nM), muscarinic M1 (513.5±99 nM) and muscarinic M2 (816.5±113) receptors (Caliper Life Sciences, Hanover, MD). The objectives of the present study was to evaluate the behavioural effects of D-84 in cocaine discrimination and self-administration assays, and to determine the effects of D-84 on cocaine self-administration in rats, in order to further evaluate its likely usefulness as a replacement therapy for cocaine dependency.

2. Materials and methods

2.1 Subjects

Adult, male, experimentally-naive Long-Evans hooded rats (Harlan Sprague-Dawley, Indianapolis, IN) were used. Subjects were individually housed in an American Association of Animal Laboratory Care-accredited facility and given *ad libitum* food (Harlan Teklad, Madison, Wisconsin) and water. For self-administration studies, ad libitum food was discontinued prior to surgery and rats were maintained at a target weight of 320 g throughout the study by adjustments in post-session feedings, which represented ~90% of their ad libitum body weight at the beginning of the study (i.e., ~ 3 months of age). For discrimination studies, rats were initially free fed, and then maintained at 85% of their calculated free feeding body weights at the commencement of training by adjustments in post-session feedings. At their training and testing weights the rats ranged in weight between 320 and 350 g.

Studies were approved by the Institutional Animal Care and Use Committee of the Virginia Commonwealth University and conformed with NIH Guidelines for Care and Use of Laboratory Animals.

2.2 Drug Discrimination Apparatus and Procedure

Rat discrimination studies were conducted in two-lever operant conditioning chambers (Med Associates Inc., St. Albans, VT) equipped with a house light and food dispenser that delivered 45 mg food pellets (Research Diets, Noyes Precision Pellets, New Brunswick, NJ). Scheduling of pellet deliveries and collection of data were accomplished by a microcomputer and associated interface (MED-PC® IV , Med Associates Inc., St. Albans, VT).

Drug discrimination training occurred during daily (M-F) 15-min experimental sessions. The rats were initially trained to press one of two levers under a fixed-ratio 1 (FR 1) schedule of reinforcement in which each lever press resulted in a pellet delivery. The response requirement was gradually increased to FR10. During the next few sessions the rats were reinforced only for pressing the alternate lever until they pressed reliably under FR10 scheduling conditions, after which drug discrimination training commenced. Rats were injected with 10 mg/kg cocaine or saline vehicle i.p., 10 min prior to the start of the session. For each rat, one lever was designated correct following drug administration and the other as correct following saline administration. The lever upon which the rats initially acquired the lever press response was designated as the vehicle-appropriate lever. Alternation of cocaine and saline injections proceeded according to a two-monthly cycle (Month #1: CSSCS, SCCSC, SCSCS, CSCSC; Month #2: SCCSS, CSCSC, CSSCC, SCSCS; in which C=cocaine S=saline). Lever pressing produced pellet delivery only on the injection-appropriate lever for that day. Incorrect presses reset the response requirement on the correct lever.

Substitution tests began once a rat met the following criteria: 1) the first completed fixed-ratio (FFR) occurred on the lever designated correct on at least eight of ten consecutive sessions; and 2) at least 80% of the total responses were emitted on the correct lever during those eight sessions. After these initial training criteria were met, testing could occur twice a week on Tuesdays and Fridays, provided that the rats completed the FFR on the correct lever during the most recent training drug and saline sessions; otherwise, a training day was administered. Test sessions were identical to training sessions except completion of the FR10 contingencies on either lever resulted in pellet delivery. Dose-response curves were collected first with cocaine (1-30 mg/kg) before substitution tests with D-84 (1-42 mg/kg) were conducted.

2.3 Self-administration Apparatus and Procedure

Self-administration tests were conducted in operant conditioning chambers housed inside individual, isolated and ventilated boxes (Med Associates Inc., St. Albans, VT). The front wall of each chamber was equipped with two retractable levers with a white stimulus light above each lever. A 5-w house light and Sonalert® tone generator were located on the rear wall of the chamber.

2.4 Surgical procedure

After acclimation to the vivarium, indwelling venous catheters were implanted into the right external jugular vein using procedures and catheters similar to that described previously (Shelton and Beardsley, 2005; Shelton et al., 2004). Rats were allowed to recover from surgery for at least 5 days before self-administration training began. Between sessions the catheters were flushed and filled with 0.1 ml of a 25% glycerol/75% sterile saline locking solution containing 250 units/ml heparin, 250 mg/ml ticarcillin, and 9 mg/ml clavulanic acid. Periodically throughout training, methohexital (1.5 mg/kg) or ketamine (5 mg/kg) (KetaThesia, Butler Animal Health Supply, Dublin, OH) was infused through the catheters to determine patency as inferred when immediate anesthesia was induced. This procedure was done infrequently (<< once per week per rat) and only when catheter patency was suspected to be compromised. Between sessions the catheters were flushed and filled with 0.1 ml of a 25% glycerol (Acros, New Jersey)/75% sterile saline locking solution containing: 250 units/ml heparin (Abraxis Pharmaceutical Products, Schaumburg, IL) and 250 mg/ml ticarcillin/9 mg/ml clavulanic acid (Timentin, GlaxoSmithKline, Research Triangle Park, NC). If during the experiment a catheter was determined to lose patency the left external jugular was then catheterized and the rat was returned to testing.

2.5 Pre-treatment tests

Cocaine self-administration training sessions were 2 h in duration and were conducted 5 days a week (M-F), unless testing had begun, and then sessions were run continuously until testing was completed. Initially, each response (fixed ratio 1, FR1) on the right-side lever resulted in delivery of a 0.5-mg/kg cocaine infusion (0.18 ml/6 sec). For the duration of the infusion, the tone sounded and the stimulus lights above both levers flashed at 3 Hz. Active (right-side) lever presses during the infusions as well as all inactive (left-side) lever presses were recorded but were without scheduled consequences.

Pre-treatment testing began once rats had met the following criteria: neither increasing nor decreasing trends in infusion numbers for 3 consecutive sessions had occurred, and the animals obtained at least 15 infusions during each session. Once these criteria were met, daily injections of vehicle were administered i.p. immediately prior to each session in order to habituate the rats to pre-session injections.

After training criteria were met, a cocaine self-administration dose-effect curve was obtained. Initially, saline was substituted for cocaine for at least three consecutive sessions. When the number of saline infusions during the third or later session of its substitution was $\leq 50\%$ the number of infusions during the most recent session of cocaine administration, the rats were returned to 0.5 mg/kg/inf cocaine self-administration. When the number of infusions of the training dose of cocaine obtained during a session was greater than the number of saline infusions during the last session of its substitution, a new dose of cocaine could then be substituted. Each dose of cocaine was substituted for one day. Between substitutions the rats were returned to 0.5 mg/kg/inf cocaine self-administration for at least one session and until the number of infusions exceeded the number obtained on the last day of saline substitution. Before each cocaine dose test session, either vehicle or a dose of D-84 was given. Pretreatment tests with vehicle preceded those with test compounds. Doses of D-84 (9.6, 17.1 and 30.4 mg/kg)

were tested across the self-administration dose-effect curve of cocaine. Self-administered doses of cocaine tested included 0.01, 0.1, 0.5 and 1 mg/kg/inf. These self-administration doses were selected based upon historical studies performed in this laboratory, and which reliably resulted in an inverted U-shape curve characterizing the number of infusions obtained as a function of dose. Pre-treatment doses of D-84 were selected based upon the ED50 value of D-84 and doses one half log unit higher and lower as determined during a previous, mouse cocaine discrimination study (Ghorai et al 2003).

2.6 Self-administration of D-84

Once pretreatment studies had been completed, other rats were trained to self-administer 0.5 mg/kg/inf cocaine in an identical fashion as described for rats used in the pretreatment study. When neither increasing nor decreasing trends in infusion numbers for three consecutive sessions had occurred, and the rats had obtained at least 15 infusions during each session, cue changes (stimulus light flashes above levers and Sonalert activations) during infusions were discontinued. Discontinuation of cue changes were initiated to permit rapid extinction of lever pressing during substitutions with doses of D-84. After a further three sessions of 0.5 mg/kg/infusion cocaine self-administration during which neither increasing nor decreasing trends in infusion numbers occurred, saline was substituted as the available infusate until the number of infusions obtained during a session was less than 50% of the mean of the most recent three cocaine sessions. Rats were then returned to 0.5 mg/kg/inf cocaine availability without cues until trends in infusion numbers did not occur across three consecutive sessions. Vehicle, and increasing doses of D-84 (0.1, 0.3, 0.56 and 1.0 mg/kg/infusion) were then substituted for three consecutive sessions each. Following tests with D-84, the rats were returned to 0.5 mg/kg/inf cocaine availability for three consecutive sessions.

2.7 Drugs

Cocaine HCl was obtained from the National Institute of Drug Abuse, and was dissolved in 0.05% saline. D-84 was synthesized according to methods described previously (Ghorai et al 2003), and was found to pass purity standards established by the American Chemical Society for journal publication with an elemental analysis of C (68.20), H (6.31), and N (2.71) similar to that predicted of C (68.35), H (6.32), and N (2.74). D-84 was solubilized in 20% β-cyclodextrin (Cavitron 82003, Cargill Food and Pharma specialists, Cedar Rapids, IA) in sterile water. During discrimination tests, D-84 was administered i.p. 20 min before the start of the test session. This pre-treatment time was chosen because previous locomotor studies indicated that peak effects on locomotor activity occurred at 20 min post-injection. Cocaine was administered 10 min before the start of discrimination studies base on previous cocaine discrimination studies conducted in this laboratory. All drugs were administered in a volume of 1.0 ml/kg body wt. when administered i.p.

2.8 Data Analysis

For drug discrimination studies, the percentage of cocaine-lever responding (%CLR) was calculated for each subject by dividing the number of lever presses emitted upon the cocaine lever by the total number of presses emitted upon both levers and multiplying this quotient by 100. Individual values of %CLR were then averaged (±S.E.M). Complete generalization to the cocaine discriminative stimulus was inferred when %CLR was ≥ 80%. Mean response rates for each test condition were calculated by dividing the total number of lever presses emitted upon both levers by the session duration (900 s) for each subject, and then these rates were averaged (±S.E.M). If a rat failed to make at least ten lever presses during a test session, its data were excluded from calculations of %CLR but were included for mean response rate determinations. This exclusion was made to prevent near-zero rates of responding from disproportionately influencing percent cocaine lever responding. ED_{50} values and their

confidence intervals (CI) were calculated for %CLR and for reducing response rates using nonlinear regression analysis.

For self-administration substitution studies, data from the last day of substitution at each dose were used in the analyses. One-way repeated measures ANOVA, followed by Dunnett's post tests, were used to compare self-infusions of drug to self-infusions of vehicle. Because during pretreatment tests one rat could not complete tests at all doses of cocaine at 9.6 mg/kg D-84 and another at 17.1 mg/kg D-84 because the catheters implanted in their remaining, catheterizable vein had lost patency, a repeated measures ANOVA could not be conducted on cocaine infusions or intake (because not all rats were tested under all conditions). Instead, non-repeated measures ANOVAs were conducted. Subsequently, Bonferroni post-tests were conducted comparing cocaine intake during pretreatments with D-84 to vehicle pretreatment. Statistical significance was assumed in all analyses if $P<0.05$. Statistical analyses and nonlinear regressions were performed using micro-computer-based software (GraphPad Prism version 5.0 for Mac OSX, GraphPad Software, San Diego, California USA).

3. Results

3.1 Effects of D-84 in cocaine discriminating rats

Cocaine completely generalized to the 10 mg/kg cocaine training dose at doses of 10 and 30 mg/kg with an ED50 (CI) value for producing cocaine-lever responding of 3.13 (1.54-6.34). When saline or D-84s vehicle were tested, near-zero levels of cocaine-lever responding occurred (Fig. 1, Upper panel). As D-84 dose increased, levels of %CLR increased until a maximum of 76% CLR occurred at a dose of 30.4 mg/kg, and complete generalization to the 10 mg/kg cocaine training dose never occurred (Fig.1 upper panel). The ED50 (±CI) for D-84 to occasion 50% CLR (CI) was 17.94 (7.23-44.50) mg/kg. Cocaine dose-dependently reduced response rates with an ED50 (CI) value of 20.39 (7.24-57.44) mg/kg. D-84 also dose-dependently reduced response rates with an ED50 (CI) value of 30.94 (12.34-77.60) mg/kg.

3.2 Self-administration of cocaine and D-84

Fig. 2 (upper panel) shows the mean numbers of infusions obtained when cocaine and D-84 were available for self-administration. The relationship between self-administered cocaine infusions and dose was characterized by an inverted U-shaped curve with peak numbers of infusions occurring at the intermediate cocaine dose of 0.1 mg/kg/infusion, which was significantly greater than saline control numbers ([$F(4,20)=9.402$, $P=0.0002$]; $q=5.635$, $P<0.05$). D-84's self-administration was also characterized by a U-shaped curve relating infusions to dose with peak numbers of infusions occurring at 0.3 mg/kg/infusion, and which were significantly greater than vehicle control numbers ([$F(4,20)=3.803$, $P=0.0186$]; $q=3.096$, $P<0.05$). Fig. 2 (lower panel) shows mean drug intake (mg/kg/2-h session) of self-administered cocaine and D-84. Mean drug intake for both cocaine and D-84 increased as a function of dose. Peak levels of D-84 (27.81±10.52) intake were nonsignificantly ($t(10)=0.3338$, $P=0.7521$) different from peak levels of cocaine (24.33±3.73) intake.

3.3 Effects of D-84 Pre-treatment on cocaine intake

Fig. 3 shows the mean number of infusions (upper panel) and mean cocaine intake (mg/kg/2-h session; lower panel) obtained following D-84 pretreatment as a function of self-administered cocaine dose. There was a significant treatment [$F(3,72)=12.34$, $P<0.0001$] and cocaine dose [$F(3,72)=52.75$, $P<0.0001$] effect. Compared to vehicle pretreatment, the 17.1 mg/kg D-84 dose reduced intake at 0.5 mg/kg cocaine ($t(9)=2.627$, $P<0.05$), and the 30.4 mg/kg D-84 did so at cocaine self-administered doses of 0.1 mg/kg ($t(10)=2.696$, $P<0.05$), 0.5 mg/kg ($t(10)+3.512$, $P<0.01$), and 1.0 mg/kg ($t(10)=4.007$, $P<0.001$).

4. Discussion

The objective of the current study was to characterize the behavioural effects of the selective DAT inhibitor, D-84, a 3-hydroxy-piperazine derivative of GBR-12935 (Ghorai et al., 2003), in order to further determine its potential as a replacement therapy for cocaine dependence. The results showed that D-84 partially generalized to the cocaine discriminative stimulus, attenuated cocaine self-administration and was self-administered at lower levels than cocaine.

Previous research has shown that binding potencies of DAT inhibitors correlate well with their reinforcing efficacy in self-administration (Ritz et al., 1987), and as a result, research into replacement therapies for cocaine dependence has focused on DAT inhibitors (Carroll et al., 2006; Katz et al., 2003). The fast onset and short duration of action of psychostimulant drugs likely contribute to their abuse liability, and it is thought that a replacement therapy with a slower onset and longer duration of action may compete with and effectively reduce craving for cocaine, but have a lower abuse potential than cocaine. This theory is based on the successful treatment of treating opiate dependence with the replacement therapy, methadone (Uchtenhagen, 2003).

There have been several studies evaluating the effectiveness of DAT inhibitors as replacement therapies for cocaine-abusing subjects, but so far none have proven unequivocally effective or have had unacceptable toxicity. GBR-12909 has a high affinity for the DAT and results in non-human primates showed that it attenuates cocaine self-administration, however clinical trials were halted during phase I due to cardiac toxicity (Stafford et al., 2000). It is possible that a more selective DAT inhibitor with less NET activity would provide the right balance of cocaine-like effects without the abuse potential of cocaine, and therefore provide a usable replacement therapy for cocaine dependence.

To that end, D-84 was synthesized and subsequently identified as one of the most selective DAT inhibitors here-to-fore disclosed (Ghorai et al., 2003). In the current study, D-84 occasioned incomplete generalization (76%) in cocaine discriminating rats. This is consistent with previously published data showing that D-84 occasions incomplete generalization (67% CLR) to cocaine in mice (Ghorai et al.,2003). This systematic replication across species of the incomplete generalization to cocaine's discriminative stimulus by D-84 strengthens the overall inference of its incomplete substitution for cocaine. Because in vitro efficacy at the DAT generally correlates well with in vivo efficacy for inhibiting DA uptake, and corresponding cocaine-like effects, it is surprising that D-84, being a potent and efficacious DAT inhibitor, did not occasion complete generalization to cocaine's discriminative stimulus. Partial generalization implies that the presence of cocaine-like interoceptive effects produced by the test compound, in this case D-84, are not sufficient to control behavior as does cocaine. There are other DAT compounds that fall into this category of being selective for DAT over serotonin or norepinephrine without producing full generalization. Benztropine is a well known cocaine analogue that binds to the DAT with similar potency to cocaine but does not produce complete generalization in cocaine discrimination studies (Newman et al., 1994). It is thought that the decrease in cocaine-like behavioural effects of benztropine analogues is likely due to the anti-muscarinic effects inherent to these compounds (Ranaldi and Woolverton, 2002). A broad (63 receptors) spectrum binding evaluation of D-84 (Caliper Life Sciences, Hanover, MD) indicated that it has low muscarinic activity (M1 Ki= 512 nM, M2 Ki= 816 nM) that unlikely limited its generalization to cocaine. D-84 also has activity at D4 receptors (Ki 28.9 nM), but it is difficult to determine how much of an influence this may have had on D-84's behavioural effects.

A potential cocaine replacement therapy likely needs to share some of the subjective effects with cocaine, and D-84 does occasion partial cocaine-lever responding. D-84's partial

generalization to cocaine's discriminative stimulus could translate into a reduction of craving for cocaine if used as a medication. D-84 was self-administered, but was less potent than cocaine. Its self-administration suggests it likely would promote patient compliance. Finally, D-84's ability to reduce self-administration of cocaine suggests that it may substitute for cocaine when given as a pretreatment. This latter suggestion, however, needs qualification somewhat by the observation that D-84 also reduced food-maintained response rates at high doses during discrimination tests, suggesting that some of the non-specific effects of D-84 could contribute to the attenuation of cocaine self-administration.

D-84 is less potent but longer acting than cocaine for stimulating locomotor activity in mice (Ghorai et al., 2003). D-84 is both less potent and efficacious than cocaine in producing cocaine's discriminative stimulus effects in mice (Ghorai et al., 2003) and in rats (present study). D-84 was also less potent than cocaine as a reinforcer in the present study. Considering D-84s general lower potency than cocaine, and observing that D-84 was self-administered at intake levels no greater than cocaine, it seems likely that peak cocaine-like effects were never obtained with D-84 during the self-administration tests. Those observations raise the possibility that D-84 has less reinforcing efficacy, and a consequent lower abuse potential, than cocaine. Additional studies, however, such as those using progressive ratio schedules and/or a behavioral economic analysis comparing D-84 with cocaine and other drugs of abuse using within-subject comparisons are needed to more definitively identify D-84s relative reinforcing efficacy.

5. Conclusions

The results of the current study show that D-84 is less potent and efficacious than cocaine in occasioning cocaine-like subjective effects, and can reduce cocaine self-administration when given as a pretreatment. Despite its lower potency, D-84 is self-administered at intake levels no greater than that of cocaine's. These results show that D-84 possesses some favorable characteristics likely desirable for a replacement therapy in treating cocaine dependency, and encourages further study for its utility as a pharmacotherapeutic.

Acknowledgments

none

8. References

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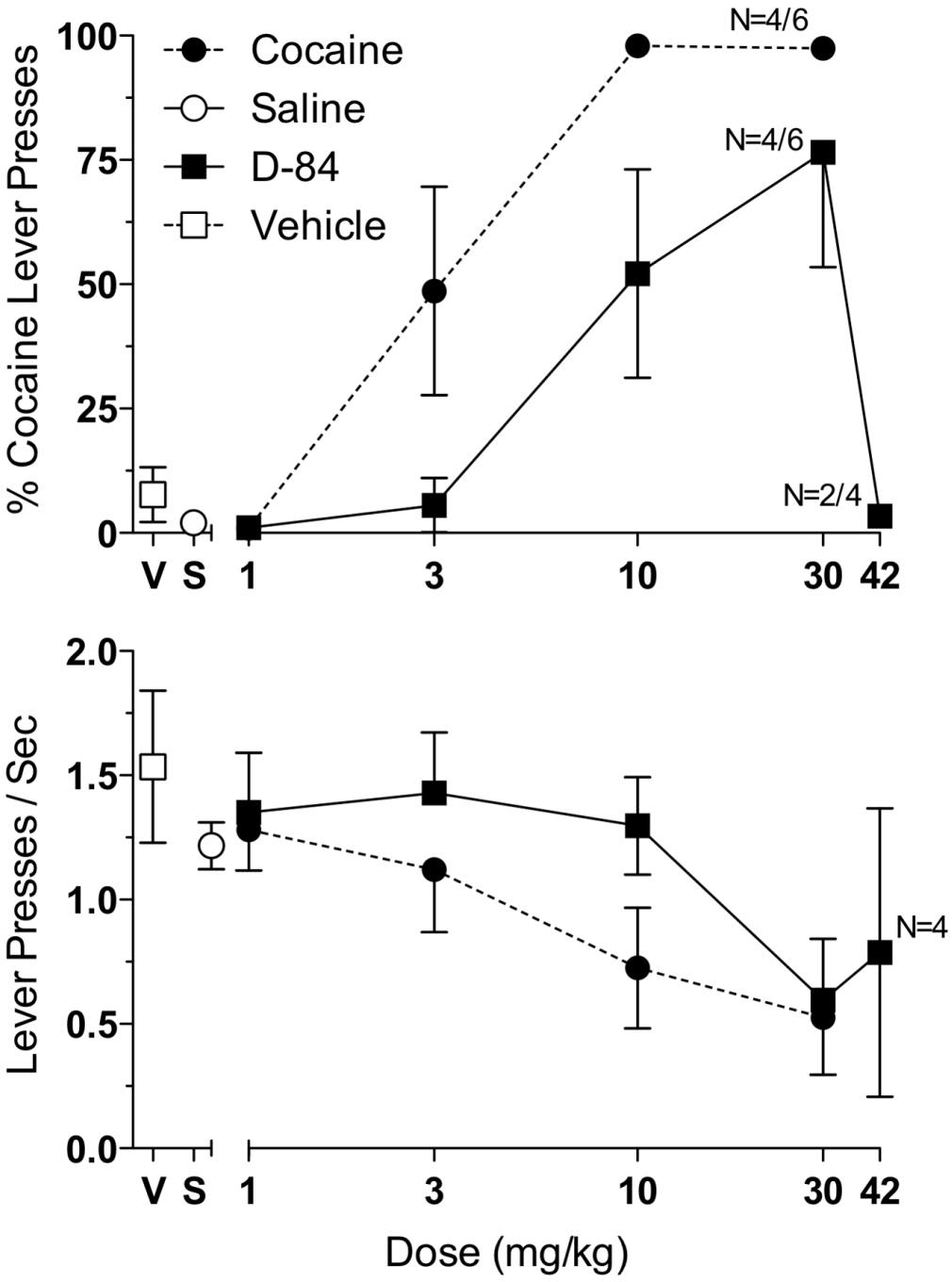

Fig. 1.

Upper panel: Effects of cocaine and D-84 dose (mg/kg) on the percentage of cocaine-lever responses in rats trained to discriminate 10 mg/kg cocaine. “V”=20% w/v cyclodextrin vehicle; ”S”=saline; filled circles=cocaine; filled squares=D-84. Each symbol represents a mean of six rats, except N=4 and 2 at 30 and 42 mg/kg, respectively, because some rats failed to meet minimum response rate criteria (see text) and their data were not included for %CLR expressions but were included for expressions of response rate. Bars represent ±S.E.M.. When ratios of N are provided at a dose the denominator indicates total rats tested at that dose, and the numerator indicates the number meeting response rate criteria. If a rat failed to meet

response rate criteria at a lower dose, it was not tested at higher doses (e.g., only four rats were tested at 42 mg/kg because two had not met response rate criteria at 30 mg/kg).

Bottom panel: Mean numbers of total lever presses per sec. Each symbol represents a mean of six rats except N=4 at 42 mg/kg because two rats failed to meet response criteria at the preceding, lower dose (i.e., at 30 mg/kg) and were not tested at higher doses (see text). Other details as described for the upper panel.

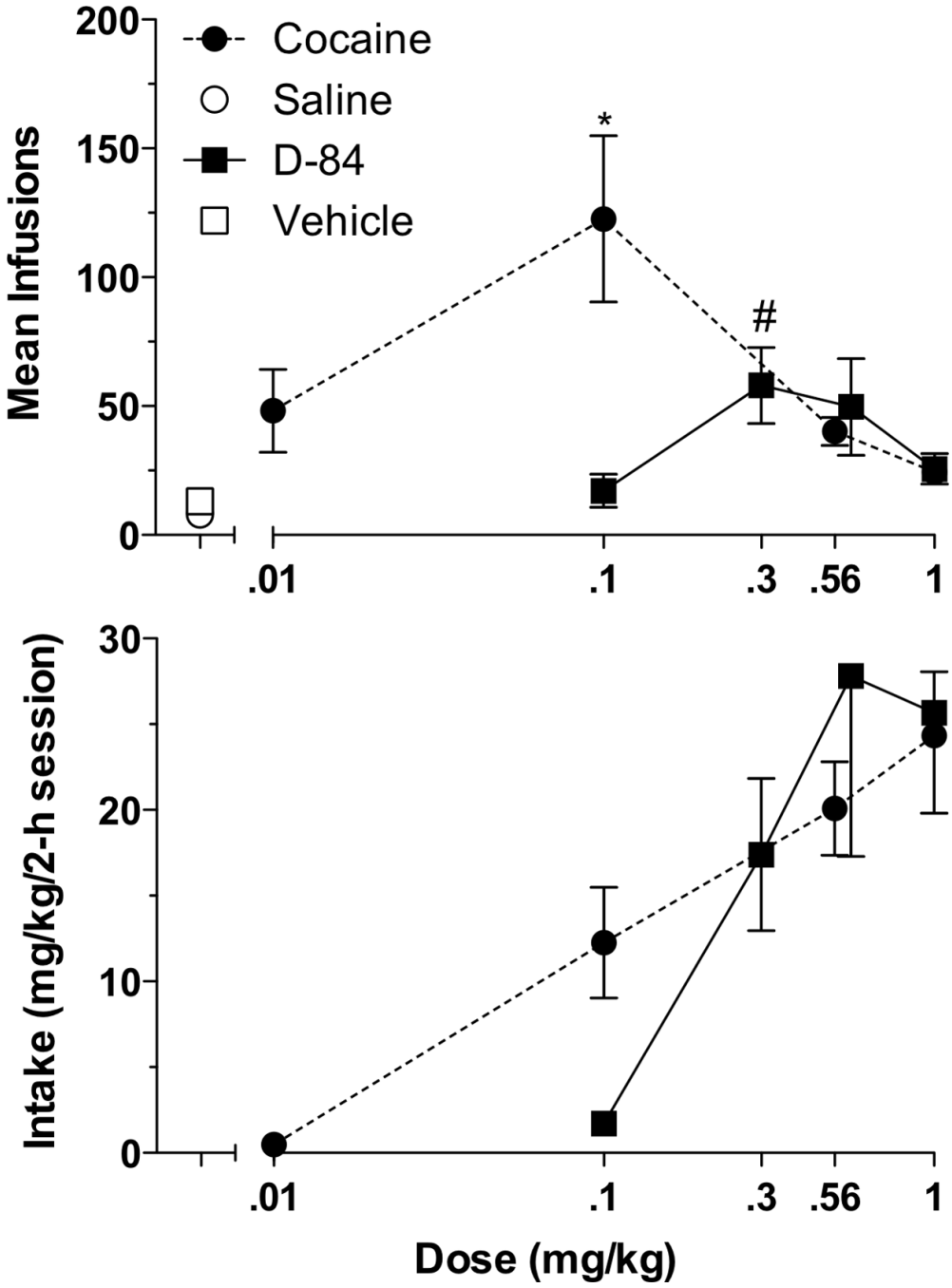

Fig. 2.

Upper panel: Mean infusions of saline (unfilled circles) and D-84s vehicle (unfilled squares), and of cocaine (filled circles) and D-84 (filled squares) obtained as a function of dose. Brackets through data points indicate ±S.E.M.. N=6 at all conditions. * = $P < 0.05$ as compared to saline; # = $P < 0.05$ as compared to vehicle.

Bottom panel: Mean drug intake (mg/kg/2-h session) as a function of dose. Other details as described for the upper panel.

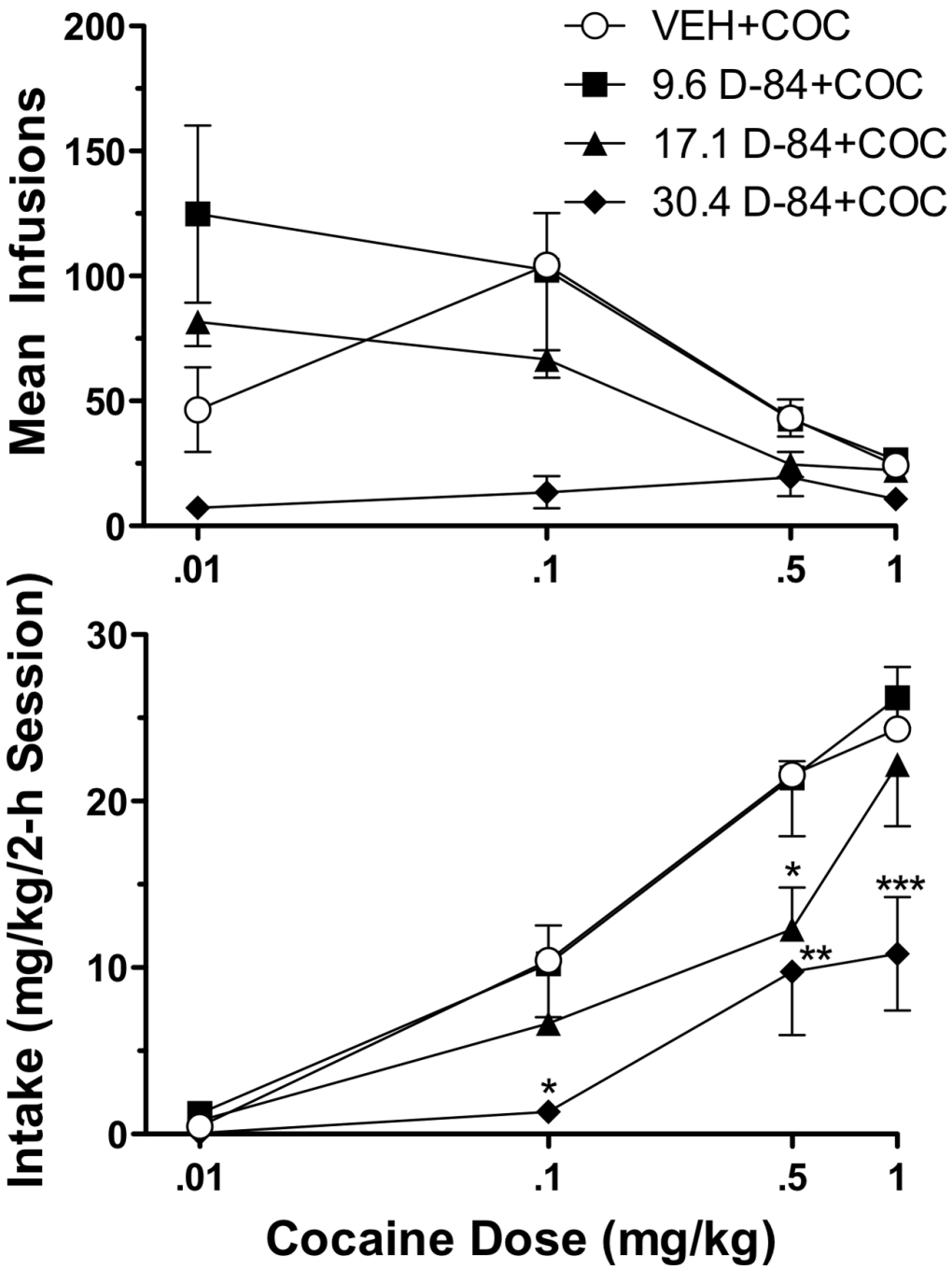

Fig. 3.

Upper panel: Mean infusions of cocaine doses of 0.01, 0.1, 0.5 and 1 mg/kg/infusion as a function of D-84 pretreatment dose. Bars through the data points indicate the S.E.M. N=5 at 9.6 and 17.1 mg/kg D-84; N=6 at 30.4 mg/kg D-84.

Bottom panel: Cocaine intake (mg/kg/2-h session) obtained as a function of D-84 pretreatment dose. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.002$ as compared to vehicle pre-treatment. Other details as in the upper panel.