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Sigma receptor agonists: Receptor binding and effects on mesolimbic dopamine neurotransmission assessed by microdialysis

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Abstract

Background—Two subtypes of sigma (σ) receptors, σ_1 and σ_2 , can be pharmacologically distinguished, and each may be involved in substance-abuse disorders. σ -receptor antagonists block cocaine place conditioning and σ -receptor agonists are self-administered in rats that previously self-administered cocaine. Self-administration has been related to increased dopamine (DA) neurotransmission for different drug classes. Actions of σ -receptor agonists on mesolimbic DA have not been fully characterized.

Methods—Receptor-binding studies assessed affinities of different σ -receptor ligands for σ -receptor subtypes, and for the DA transporter; effects on DA transmission in the rat nucleus accumbens shell were assessed using *in-vivo* microdialysis.

Results—Cocaine (0.1–1.0 mg/kg i.v.), the non-selective $\sigma_{1/2}$ -receptor agonist DTG (1.0–5.6 mg/kg i.v.), and the selective σ_1 -receptor agonist PRE-084 (0.32–10 mg/kg i.v.) dose-dependently increased DA, with maxima of about 275, 150, and 160%, respectively. DTG-induced stimulation of DA was antagonized by the nonselective $\sigma_{1/2}$ -receptor antagonist, BD 1008 (10 mg/kg i.p.), and by the preferential σ_2 -receptor antagonist SN79 (1–3 mg/kg i.p.), but not by the preferential σ_1 -receptor antagonist, BD 1063 (10–30 mg/kg i.p.). Neither PRE-084 nor cocaine was antagonized by either BD1063 or BD1008.

Conclusions—Stimulation of DA by σ -receptor agonists in a brain area involved in the reinforcing effects of cocaine was demonstrated. The effects appear to be mediated by σ_2 -receptors rather than σ_1 -receptors. However σ -receptors are not likely involved in mediating the

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acute cocaine- and PRE-084-induced stimulation of DA transmission. Different mechanisms might underlie the dopaminergic and reinforcing effects of σ -receptor agonists suggesting a dopamine-independent reinforcing pathway that may contribute to substance-abuse disorders.

Keywords

abuse liability; addiction; cocaine; dopamine microdialysis; nucleus accumbens; reinforcing effects; sigma receptors

Introduction

Sigma (σ) receptors, initially proposed as opioid (1) and later phencyclidine (PCP) receptors (2), were demonstrated to represent a unique binding site in the mammalian brain and peripheral organs (3–5), and are expressed throughout the central nervous system. The σ -receptor system has been implicated in a variety of physiological functions and disease states (6).

Pharmacological and structural studies distinguished two receptor subtypes: sigma 1 (σ_1) and sigma 2 (σ_2) (5,7). The σ_1 receptor was cloned and characterized as a 29 kDa single polypeptide having no homology with any other known mammalian proteins (8). In contrast, the σ_2 receptor is an 18–21 kDa protein that has not yet been cloned (9). σ_1 receptors possess two transmembrane domains and have been localized subcellularly at the endoplasmic reticulum. It has been suggested that, after stimulation by sigma ligands, σ_1 receptors translocate to the plasma membrane (10).

Several classes of compounds, including neurosteroids, neuroleptics, dextrobenzomorphans, and psychostimulants, such as methamphetamine and cocaine, have been shown to bind to σ receptors (11,12). The psychomotor stimulant and reinforcing effects of cocaine are primarily mediated through stimulation of dopamine (DA) neurotransmission by inhibiting DA reuptake. However, its moderate affinity for σ receptors (13–15), suggests that cocaine-induced effects could involve actions at σ_1 - and/or σ_2 -receptor subtypes (16). Consequently, studies have examined the effects of σ -receptor ligands on various effects of cocaine related to its abuse (see reviews by 11, 17, 18). For example, σ -receptor antagonists blocked the locomotor-stimulant effects of cocaine (19–22), and the development of cocaine-induced locomotor sensitization (22,23). Further, σ -receptor antagonists attenuated cocaine-induced place conditioning (24), though these compounds failed to substantially alter cocaine self-administration (25,26), indicating that σ receptors are not directly involved in the direct reinforcing effects of cocaine. However, the preferential σ_1 -receptor antagonist BD 1047 attenuated the reinstatement of cocaine-reinforced behavior induced by cocaine-related stimuli, suggesting that σ receptors might be involved in brain mechanisms and behavioral activities that trigger cocaine use. Finally, several σ -receptor antagonists can block or attenuate acute cocaine-induced toxicities, such as convulsions and lethality (13). Together, these studies suggest that σ_1 receptors participate in the mechanisms of action of cocaine, and that σ -receptor antagonists might be potentially useful as medications for treating cocaine abuse, dependence, and overdose (24,27).

Recently Hiranita and colleagues (26) showed reinforcing effects of σ -receptor agonists in rats trained to self-administer cocaine. Reinforcing effects of most drugs of abuse appear to be mediated by stimulation of DA transmission, and it has been repeatedly shown that the acute effects of drugs abused by humans at doses able to maintain self-administration in rodents, preferentially or selectively increase DA transmission in the nucleus accumbens (NAc) shell as compared to the NAc core or dorsal striatum (28–30). Increases in DA transmission induced by non-selective σ -receptor agonists have been reported (31,32),

though contrasting results and sometime biphasic effects have been reported after systemic or local intrastratial administration (33–36). Thus, the goal of the present study was to test the hypothesis that intravenous (i.v.) administration of σ -receptor agonists would increase DA transmission in the NAc shell. To this end, rats were implanted with microdialysis probes in the NAc shell and effects of various i.v. doses of DTG, a non-selective σ -receptor agonist, and PRE-084, a selective σ_1 -receptor agonist, on extracellular DA levels were compared to the effects of i.v. cocaine. In addition, the effects of the σ -receptor agonists as well as cocaine were studied in combination with σ -receptor antagonists to confirm the pharmacological specificity of the effect. σ -receptor ligands were also studied in binding experiments to test their affinities for σ_1 - and σ_2 -receptor subtypes as well as the DA transporter (DAT), the main pharmacologic target of cocaine.

METHODS

Sigma 1 and sigma 2 Receptor Binding

Frozen whole guinea-pig brains (minus cerebellum) were used and processed as already published (37)(see also Supplement for complete details). The guinea pig brains were preferred over rat brains due to their use as a standard for sigma receptor binding because of the relatively higher density of those receptors in that tissue compared to rat (38). Ligand binding experiments were conducted for σ_1 -receptor studies with 3 nM [3 H]pentazocine (specific activity 28 Ci/mmol) and 8.0 mg tissue. Nonspecific binding was determined using 10 μ M haloperidol. For σ_2 -receptor studies, each tube contained 3 nM [3 H]DTG (specific activity 48 Ci/mmol), 200 nM (+)-pentazocine, and 8.0 mg tissue. Nonspecific binding was determined using 100 μ M haloperidol.

DAT Binding

Brains from male Sprague-Dawley rats weighing 200–225 g (Taconic Labs) were used, and experiments were carried out as published previously (39). Binding was assessed with 0.5 nM [3 H]WIN 35428 (specific activity 84 Ci/mmol) and 1.0 mg striatal tissue. Nonspecific binding was determined using 0.1 mM cocaine HCl.

In-vivo microdialysis studies

Male Sprague-Dawley rats (Charles River, Germantown, MD, USA), weighing 275 to 350 g served as subjects, and all methods were as described previously (30,40–42) (see details in Supplement). Rats were doubly housed in a temperature- and humidity-controlled room maintained on a 12-h light/dark cycle (lights on: 07:00–19:00 hour) and had free access to food and water. Experiments were conducted during the light phase.

Surgical procedures were conducted under a mixture of ketamine and xylazine (60.0 and 12.0 mg/kg ip, respectively) anesthesia. Rats were first implanted with a silastic catheter into the external jugular vein, with the catheter exiting the skin at the back between the shoulders. Rats were then placed in a stereotaxic apparatus, and implanted with concentric dialysis probes aimed at the NAc shell [uncorrected coordinates from the rat brain atlas of Paxinos and Watson (43): Anterior = +2.0 mm from bregma, Lateral = $\pm$ 1.0 mm from bregma, Vertical = –7.9 mm from dura,]. The histological confirmations of probe placement are included in the Supplement.

Experiments were performed on freely moving rats, about 22–24 hours after probe implant. Dialysate was sampled every 10 min and immediately analyzed. After stable DA values (less than 10% variability) were obtained for at least three consecutive samples (after about 1–2 hours), rats were treated with one dose of one of the test drugs (cocaine, DTG, PRE-084, or saline), or with an antagonist (BD 1008, BD 1063, or SN79) followed 30 (or 10

for SN 79) min later by saline or one of the test drugs. Different groups of naïve rats were used for each different treatment. In another series of experiments in different groups of naïve rats, after obtaining stable DA levels, microdialysis probes were perfused with calcium-free Ringer's solution for 60 min before i.v. injections of DTG or PRE-084 (44,45).

Dopamine was detected in dialysate samples by HPLC coupled with a coulometric detector (5200a Coulochem II, or Coulochem III, ESA). The average basal DA values in dialysates from the NAc shell in the present experiments were 42.3 ± 1.0 fmoles ($\pm$ S.E.M.) in a 10 μ l sample, $n=171$. No significant differences were found in basal DA concentrations from the different experimental groups (ANOVA, $F(30,140)=0.516$, $P=0.98$).

Drugs

The following were used: (–)-cocaine hydrochloride (0.1–1.0 mg/kg i.v.), the mixed $\sigma_{1/2}$ R agonist, DTG (1.0–5.6 mg/kg i.v.), the selective σ_1 R agonist, PRE-084 (0.32–5.6 mg/kg i.v.), the preferential σ_1 R antagonist, BD 1063 (10–30 mg/kg ip), the mixed $\sigma_{1/2}$ R antagonist, BD 1008 (10–30 mg/kg ip), and the preferential σ_2 R antagonist, SN79 (1–3 mg/kg i.p. (synthesized at the Department of Medicinal Chemistry at the University of Mississippi, (18,46). Chemical names, sources of the compounds, their preparation and dose selection rationale are provided in the Supplement.

Data analysis

Data from the in vitro binding studies were fit by nonlinear regression using GraphPad Prism 5.03 (GraphPad Software, San Diego California USA). The fits of data for DTG displacement of [3 H]DTG were globally fit to one- or two-site homologous competition models. Because those data were better fit (F test) to a two-site model, competition studies of all drugs tested for displacement of [3 H]DTG were compared for global fits to both one- and two-site models for heterologous competition, with data for the best fit reported. The fits of displacement data against the other ligands were consistent with a single site model, and therefore single affinities are reported.

Results of in-vivo microdialysis studies were expressed as a percentage of basal DA values, which were calculated as means of the three consecutive samples preceding the test drug or saline injection, with results presented as group means ($\pm$ S.E.M.). Statistical analysis (Statistica software, Stat Soft, Tulsa, OK) was carried out using one-, two-, or three-way ANOVA for repeated measures over time, with drug dose, time, and antagonist pretreatments as factors. Significant results were followed by post-hoc Tukey's tests. Changes were considered significant when $p < 0.05$.

RESULTS

In vitro binding experiments

As has been previously reported (47,48), DTG and PRE-084 had high affinity for σ receptors. The homologous competition of DTG modeled better for two than one binding site (Table 1). The high affinity binding K_d value of 21.9 nM is comparable to that reported previously for σ_2 receptors in the papers cited above, and indicates a two- to three-fold selectivity for σ_2 over σ_1 receptors, as also reported previously. In contrast, PRE-084 had a high affinity for σ_1 receptors (53.2 nM), and was approximately 600-fold selective for this site over σ_2 receptors. Among the σ -receptor antagonists tested, BD 1008 had the highest affinity for both σ_1 receptors and σ_2 receptors; however, this compound was the least selective of the antagonists tested. The σ -receptor antagonist BD 1047 had approximately the same affinity for σ_1 receptors as BD 1008 did but had lower affinity at σ_2 receptors. BD 1063 had lower affinity at both σ_1 receptors and σ_2 receptors but was the most selective of

the three for the σ_1 receptor. None of the compounds except cocaine had high affinity for the DAT, and that affinity was approximately 70-fold greater than its affinity for the σ_1 receptor (Table 1).

In vivo microdialysis experiments

Administration of cocaine (0.1–1.0 mg/kg, i.v.) significantly increased extracellular levels of DA within the first 10 min after injection to values approximating 275% of baseline levels at the highest dose tested. [Two-way ANOVA gave main effects of cocaine dose: $F(2,16)=3.73$, $p<0.05$, time: $F(6,96)=17.8$, $p<0.001$; and dose-by-time interaction: $F(12,96)=3.53$, $p<0.001$]. Stimulation of DA levels was transient, and levels of DA approached basal levels within 50 min after injection of the highest dose (Figure 1A). The effects of cocaine obtained in the first 30 min after injection were significant, and directly related to dose (Figure 1B). [Two-way ANOVA showed significant main effects of dose: $F(2,16)=4.58$, $p<0.05$; time: $F(3,48)=22.7$, $p<0.001$; and dose-by-time interaction: $F(6,48)=4.14$, $p<0.001$].

Administration of the non-selective σ -receptor agonist DTG (1.0–5.6 mg/kg i.v.) transiently and significantly increased DA levels, with values returning to the non-stimulated baseline levels within approximately 45 min after administration (Figure 2A). [Two-way ANOVA gave significant main effects of dose: $F(2,18)=6.76$, $p<0.01$; time: $F(6,108)=5.58$, $p<0.001$; and dose-by-time interaction: $F(12,108)=4.15$, $p<0.001$]. Maximal effects of the highest (5.6 mg/kg) dose were observed within 10 min after injection whereas the maximal effects of 3.2 mg/kg were observed at the 20 min sampling time. The effects of DTG in the first 30 min, as with cocaine, show a linear increase over the dose range studied (Figure 2B). Maximal increases in DA concentration were obtained at 5.6 mg/kg which was approximately 50% of the effect obtained with the highest dose of cocaine studied. At higher doses, DTG produced signs of acute toxicity. At the doses tested, DTG was less potent than cocaine. The selective σ_1 -receptor agonist, PRE-084 (0.32–10 mg/kg i.v.), also increased DA levels and the effect was significant at the highest dose tested (Figure 2C). [Two-way ANOVA gave significant main effects of dose: $F(4,17)=3.94$, $p<0.02$, time: $F(9,153)=5.96$, $p<0.001$; and dose-by-time interaction: $F(36,153)=4.32$, $p<0.001$]. The maximal increase in DA levels obtained with the 10 mg/kg dose of PRE-084 was about 170% of basal values at 10 min after administration (Figure 2D).

The preferential σ_1 -receptor antagonist, BD 1063 (10–30 mg/kg i.p.), administered alone induced a small, transient (<30 min) non-significant increase in DA levels of about 30%, and a later small decrease in DA levels to about 80% of baseline (Figure 3A and B, open squares). A 30-min pretreatment with the preferential σ_1 -receptor antagonist BD 1063 did not significantly alter the time course of 5.6 mg/kg of DTG (Figure 3A and 3B), nor its maximal effects (Figure 3C). [Three-way ANOVA gave significant main effects of DTG treatment: $F(1,30)=7.07$, $p<0.05$, time: $F(6,180)=9.40$, $p<0.001$; and the DTG-treatment-by-time interaction: $F(6,180)=11.4$, $p<0.001$]. In addition non-significant effects of BD1063 pretreatment: $F(2,30)=1.39$; and the interactions of DTG-treatment-by-BD1063-pretreatment: $F(2,30)=0.035$; BD1063-pretreatment-by-time: $F(12,180)=1.05$; and DTG-treatment-by-BD1063-pretreatment-by-time: $F(12,180)=0.420$ were obtained].

The non-selective σ -receptor antagonist, BD 1008 (10 mg/kg i.p.) had no immediate effects of its own (Figure 4A and B, open squares), though modest decreases in DA levels were obtained over the 2 hr observation period to levels approximating 75% of basal control values at 2 hr after injection. BD 1008 pretreatment significantly attenuated the effects of 5.6 mg/kg of DTG (Figure 4A) and completely blocked the effects of 3.2 mg/kg of DTG (Figure 4B, C). [Three-way ANOVA gave main effects of DTG treatment: $F(2,31)=3.39$, $p<0.05$; BD1008-pretreatment: $F(1,31)=15.5$, $p<0.001$; time: $F(6,186)=10.0$, $p<0.001$; and

the interactions of DTG-treatment-by- time: $F(12,186)=2.97$, $p<0.001$; BD1008-pretreatment-by-time: $F(6,186)=4.20$, $p<0.001$; DTG-by-BD1008-by-time: $F(12,186)=1.90$, $p<0.05$; though a non significant interaction of DTG-treatment-by-BD1008-pretreatment: $F(2,31)=0.103$].

The preferential σ_2 -receptor antagonist, SN79 (18,46), administered alone (1–3 mg/kg i.p.) did not significantly modify extracellular levels of DA up to 100 min after its administration (Figure 5A, B). However, SN79 pretreatment dose-dependently and significantly antagonized the effects of 3.2 and 5.6 mg/kg of DTG (Figure 5A, B). [Three-way ANOVA gave significant main effects of DTG: $F(2,32)=3.72$, $p<0.05$; SN79-pretreatment: $F(1,32)=12.4$, $p<0.005$; time: $F(3,96)=10.9$, $p<0.001$; and the interactions of DTG-treatment-by-time: $F(6,96)=5.93$, $p<0.001$; SN79-pretreatment-by-time: $F(3,96)=2.80$, $p<0.05$; and DTG-treatment-by-SN79-pretreatment-by-time: $F(6,96)=2.50$, $p<0.05$. The interaction of DTG-by-SN79-pretreatment was not significant: $F(2,32)=2.810$, $p=0.07$]. Over the range of DTG doses studied, 1.0 mg/kg of SN79 significantly antagonized the effects of DTG (Figure 5C).

A 30-min pretreatment with the preferential σ_1 -receptor antagonist, BD 1063 (10 mg/kg i.p.), had no significant effects of its own and did not significantly modify PRE-084-induced stimulation of DA transmission in the NAc shell (Figure 6A). [Two-way ANOVA gave a main effect of time: $F(9,54)=10.181$, $p<0.001$; but no significant effects of BD1063-pretreatment: $F(1,6)=0.025$; or BD1063-pretreatment-by-time interaction: $F(9,54)=0.517$]. Similarly, the non-selective σ -receptor antagonist BD 1008 (10–30 mg/kg i.p.) did not significantly modify PRE-084-induced stimulation of DA transmission in the NAc shell (Figure 6, panel B). [Two-way ANOVA gave a significant main effect of time: $F(9,81)=26.775$, $p<0.001$; but no significant effects of BD1008-pretreatment: $F(2,9)=0.971$, or BD1008-pretreatment-by-time interaction: $F(18,81)=0.498$].

As with PRE-084, the effects of cocaine on stimulation of DA transmission were not significantly modified by pretreatments with either 10 mg/kg of BD 1063 or BD 1008 administered 30 min before (Figure 7, panels A and B). [Two-way ANOVAs conducted individually for each drug interaction gave significant main effects of time $F(9,99)=19.609$, $p<0.001$ (BD1063 and cocaine) or $F(9,99)=11.357$, $p<0.001$ (BD1008 and cocaine), but non significant effects of BD 1063 pretreatment: $F(1,11)=0.028$; and its interaction with time: $F(9,99)=0.595$; or BD 1008 pretreatment: $F(1,11)=0.001$; and its interaction with time: $F(98,99)=0.568$].

Local perfusion of the NAc shell with a calcium-free Ringer's solution delivered by the microdialysis probe markedly decreased extracellular DA levels compared to basal levels collected with normal Ringer's solution (Figure 8A, B). Decreases were apparent from the first sample after treatment, and the maximal decrease was to about 15–20% of basal control values obtained with normal Ringer's solution (Figure 8A, B). Administration of vehicle (not shown), DTG (5.6 mg/kg), or PRE-084 (10 mg/kg) 60 min after the start of local perfusion with calcium-free Ringer's solution did not significantly modify the low extracellular levels of DA (Figure 8A, B). [Two-way ANOVAs conducted individually for each drug gave significant main effects of time $F(14,84)=75.0$, $p<0.001$ (DTG) or $F(14,84)=106$, $p<0.001$ (PRE-084), but non-significant effects of DTG-treatment: $F(1,6)=0.525$; and its interaction with time: $F(14,84)=0.760$; or PRE-084-treatment: $F(1,6)=0.655$; and its interaction with time: $F(14,84)=1.73$].

DISCUSSION

A recent study with rodents on self-administration of the σ -receptor agonists, DTG and PRE-084 (26), prompted the present study of the effects of these drugs on DA levels in the NAc shell. The goal of the present study was to assess whether the effectiveness of DTG and PRE-084 in maintaining self-administration behavior would correspond with an increase in DA neurotransmission in the NAc shell after acute administration. This correspondence has been found with most drugs abused by humans (28–30) and is considered a biological substrate for drug abuse psychiatric disorders (49–51). We observed that acute i.v. administration of DTG, a non-selective σ -receptor agonist, as well as the selective σ_1 -receptor agonist, PRE-084, dose-dependently increased DA levels in the NAc shell. We found no previously published reports on the effects of selective σ_1 -receptor agonists, like PRE-084, on DA transmission in limbic areas. Contrasting effects of non selective σ -receptor agonists, like DTG, on DA neurotransmission in striatal areas have been reported, and inconsistency of the results shown in those studies may be related to procedural differences, for example, to local versus systemic administration of drugs, or anaesthetized versus awake animals (33–36).

The present study extends the validity of the correlation between stimulation of DA levels and maintenance of self-administration behavior to σ -receptor agonists, like DTG and PRE-084. However, although the effects of the non-selective agonist, DTG, on DA transmission were obtained within a range of doses that approximated the doses per injection that maintained self-administration in rats (26), the doses of the selective σ_1 -receptor agonist, PRE-084, that significantly increased DA levels were about 10 to 30 times higher than the doses per injection in the self-administration study. In addition, PRE-084 was about threefold *more* potent than DTG in the self-administration study (26) whereas it was *less* potent than DTG in increasing DA levels in the present study, suggesting that different mechanisms might underlie the dopaminergic and reinforcing effects of these σ -receptor agonists. To better understand the mechanisms underlying these differences, studies of antagonism were conducted with several σ -receptor antagonists.

The previously reported preferential σ_1 -receptor antagonist effects of BD 1063 (13) were confirmed in the present binding studies. When tested in microdialysis studies, BD 1063, up to 30 mg/kg, failed to antagonize the effects of any dose tested of DTG, or PRE-084, and cocaine. The lack of antagonism by the preferential σ_1 -receptor antagonist BD 1063 suggests that σ_1 receptors are not involved in the acute effects of cocaine, PRE-084, and DTG on DA levels in the NAc shell. In contrast, the relatively non-selective $\sigma_{1/2}$ -receptor antagonist BD 1008 (20) significantly antagonized the acute effects of the non-selective $\sigma_{1/2}$ -receptor agonist DTG on DA transmission in the NAc shell.

In the present binding studies, BD 1008 was characterized as the least selective of the compounds assessed for affinity at σ -receptor subtypes, but nonetheless had higher affinity for σ_1 than σ_2 receptors. We therefore examined the antagonism of the effects of DTG by the preferential σ_2 -receptor antagonist SN79 (18,20,46). As with BD 1008, the DTG effects were antagonized by this novel preferential σ_2 -receptor antagonist. Thus the results with SN79 confirm the results with BD 1008 suggesting again that the effects of DTG on DA levels in the NAc shell are due to its effects on σ_2 receptors.

In contrast to the effects obtained with DTG, the effects of the selective σ_1 -receptor agonist PRE-084 were antagonized by neither of the σ -receptor antagonists examined (BD 1008 and BD 1063). Because of the high affinity and selectivity for σ_1 receptors and the very low affinity for the DAT that we found for PRE-084 in binding studies, we therefore tested the possibility that its effects on DA levels were the result of a non-specific DA-releasing action

of the drug that was not related to a physiological activation of the DA system. When the NAc shell was perfused with a calcium-free Ringer's solution through the microdialysis probes, neither σ -receptor agonist PRE-084 nor DTG effectively increased DA levels, suggesting that the increase in DA was the result of a physiological synaptic activity resulting in a vesicular, calcium-dependent DA release (45). Thus, the mechanism for the high-dose effects of PRE-084 on DA levels is not known at this time, but appears to be independent of its actions at σ receptors and unlike the DAT-mediated actions of cocaine. Further, the high selectivity of PRE-084 for σ_1 receptors, which has not previously been reported, is consistent with a conclusion that σ_2 receptors mediate the effects of the non-selective σ -receptor agonist DTG on DA, and suggests that σ_1 receptors are minimally involved in this effect, if at all.

Although the effects of DTG on DA levels appear mediated by σ receptors, the effects of cocaine do not, as neither of the σ -receptor antagonists tested (BD 1008 and BD 1063) altered the acute effects of cocaine on extracellular DA levels. Cocaine has approximately 70-fold higher affinity for the DAT than for σ receptors, and among σ receptors has selectivity for σ_1 over σ_2 receptors. As the effects of the selective σ_1 -receptor agonist PRE-084 indicate little involvement of σ_1 receptors in its effects on DA, the present results suggest that the effects of cocaine on extracellular DA are minimally influenced, if at all, by its affinity for σ receptors. Thus, although the acute effects of DTG and PRE-084 on DA transmission appear on first blush to be in agreement with their reinforcing effects, their effects on DA are not mediated by similar mechanisms. Further, both σ -receptor antagonists BD 1008 and BD 1063 blocked the self-administration of σ -receptor agonists (26), indicating that their reinforcing effects are mediated by σ receptors. Thus, previous and present results raise questions about the role of DA transmission in the reinforcing effects of σ -receptor agonists.

In conclusion, the present study shows that σ -receptor agonists can stimulate DA transmission in the NAc shell, a brain area that has been involved in the mediation of reinforcing effects of drugs abused by humans. σ_2 receptors appear to be primarily involved in the DA stimulating effects of DTG, which may underlie the previously reported reinforcing effects of σ -receptor agonists (26). Further, the present experiments excluded a direct involvement of σ -receptors in the acute DA-stimulating effects of cocaine and PRE-084. Finally, the present results, along with previous findings that both DTG and PRE-084 have reinforcing effects (26) suggest a dopamine-independent reinforcing pathway that may contribute to substance-abuse disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Animals used in the study were experimentally naive at the start of experiments. The animal housing facilities were fully accredited by AAALAC International and all procedures were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the NIDA Intramural Research Program and the 1996 National Research Council and Guide for care and use of laboratory animals.

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List of nonstandard abbreviations

DA	dopamine
σ	sigma
i.v	intravenous
NS	non-significant
NAc	nucleus accumbens
PCP	phencyclidine
S.E.M	standard error of the mean

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Figure 1.

Effects of i.v. administration of cocaine (0.1–1.0 mg/kg) on DA levels in dialysates from the NAc shell in rats. Panel A shows the time course of effects of different cocaine doses; panel B shows the average change in DA levels, related to cocaine doses, obtained in the first 30 min after cocaine injection. Results are means, with vertical bars representing S.E.M., of the amount of DA in 10-min dialysate samples, expressed as percentage of basal values (panel A) or of the average of DA values during the first 30 min after cocaine injection calculated as percentage of basal values (panel B). Basal DA values (fmol/sample) and group size (n) were: 36.9 ± 6.4 (4), 45.5 ± 6.5 (8), and 44.8 ± 6.8 (7) for cocaine 0.1, 0.32, and 1.0 mg/kg, respectively. $*=p < 0.05$ compared to corresponding basal DA values.

Figure 2.

Effects of i.v. administration of the non-selective $\sigma_{1/2}$ -receptor agonist DTG (1–5.6 mg/kg), and the selective σ_1 -receptor agonist PRE-084 (0.32–10.0 mg/kg), on DA levels in dialysates from the NAc shell in rats. Panels A and C show the time course of the administration of different doses of DTG and PRE-084, respectively; panels B and D show the changes in DA levels, related to drug doses, obtained during the first 30 min after DTG or PRE-084 injections, respectively. Basal DA values (fmoles/sample) and group size (n) were: 41.3 ± 5.4 (8), 42.2 ± 6.4 (7), and 52.5 ± 3.0 (6) for DTG 1.0, 3.2, and 5.6 mg/kg, respectively; and were: 51.9 ± 7.5 (4), 45.5 ± 5.1 (5), 43.4 ± 9.8 (5), 40.0 ± 3.6 (4), and 35.8 ± 6.9 (4) for PRE-084 0.32, 1.0, 3.2, 5.6, and 10 mg/kg, respectively. See figure 1 legend for other details.

Figure 3.

Effects of pretreatments with BD 1063 (10–30 mg/kg i.p.), a preferential σ_1 -receptor antagonist, on DTG-induced stimulation of DA levels in dialysates from the NAc shell in rats. Panels A and B show the time course of effects of BD 1063 injected 30 min before the most effective dose of DTG, 5.6 mg/kg i.v.; panel C shows the same data expressed as the average change in DA levels obtained in the first 30 min after DTG injection. Basal DA values (fmol/sample) and group size (n) were: 44.6 ± 7.9 (8), and 39.5 ± 2.5 (6) for BD 1063 10 mg/kg + saline or + DTG 5.6 mg/kg, respectively, and 47.9 ± 2.6 (7), and 47.3 ± 1.6 (5), for BD 1063 30 mg/kg + saline or + DTG 5.6 mg/kg, respectively. See figure 1 legend for other details.

Figure 4.

Effects of pretreatment with BD 1008, a non-selective $\sigma_{1/2}$ -receptor antagonist (10mg/kg i.p.), on DTG-induced stimulation of DA levels in dialysates from the NAc shell in rats. Panels A and B show the time course of 10 mg/kg i.p. of BD 1008 effects on DA levels stimulated by different doses of DTG, 5.6 and 3.2 mg/kg i.v., respectively. BD 1008 was administered 30 min before DTG; panel C shows the same data expressed as the average change in DA levels, related to DTG doses, obtained during the first 30 min after DTG administration. Basal DA values (fmoles/sample) and group size (n) were: 32.3 ± 7.0 (6), 49.5 ± 7.9 (6), and 49.0 ± 8.1 (7) for BD 1008 10 mg/kg + Saline, BD 1008 10 mg/kg + DTG 5.6 mg/kg, and BD 1008 10 mg/kg + DTG 3.2 mg/kg, respectively. #= $p < 0.05$

compared with the corresponding time point of administration of BD 1008 10 mg/kg + DTG 5.6 mg/kg (panel A and C), or BD 1008 10 mg/kg + DTG 3.2 mg/kg (panel B). See figure 1 legend for other details.

Figure 5.

Effects of pretreatment with SN79, a preferential σ_2 -receptor antagonist (1–3 mg/kg i.p.), on DTG-induced stimulation of DA levels in dialysates from the NAc shell in rats. Panel A shows the time course of 1–3 mg/kg i.p. of SN79 effects on DA levels stimulated by DTG, 5.6 mg/kg i.v., injected 10 min after SN79, while panel B shows the effects of SN79 pretreatment on DA levels stimulated by DTG, 3.2 mg/kg i.v., under the same experimental conditions. Panel C shows the same data expressed as the average change in DA levels, related to DTG doses, obtained during the first 30 min after DTG administration. Basal DA values (fmol/sample) and group size (n) were: 37.5 ± 4.1 (5), 28.5 ± 7.0 (6), 51.9 ± 9.9 (6), 50.0 ± 4.6 (6), and 42.3 ± 4.2 (6) for SN79 3 mg/kg + saline, SN79 3 mg/kg + DTG 5.6 mg/kg, SN79 1 mg/kg + saline, SN79 1 mg/kg + DTG 5.6 mg/kg, and SN79 1 mg/kg + DTG 3.2 mg/kg, respectively. #= $p < 0.05$ compared with the corresponding time point of administration of SN 79 + DTG 5.6 mg/kg (panels A and C), or SN79 + DTG 3.2 mg/kg (panel B). See figure 1 legend for other details.

Figure 6.

Effects of pretreatment with BD 1063 (10 mg/kg i.p.), a preferential σ_1 -receptor antagonist, and BD 1008 (10–30 mg/kg i.p.), a non-selective $\sigma_{1/2}$ -receptor antagonist, on PRE-084-induced stimulation of DA levels in dialysates from the NAc shell in rats. Panel A shows the time course of effects of a 30-min pretreatment dose, 10 mg/kg ip, of BD 1063 on DA levels stimulated by PRE-084, 10 mg/kg i.v.; Panel B shows the time course of the effects of 10 and 30 mg/kg ip doses of BD 1008 on DA levels stimulated by PRE-084, 10 mg/kg i.v. Basal DA values (fmol/sample) and group size (n) were: 38.8 ± 2.2 (4), 38.5 ± 9.2 (4), and 33.1 ± 6.4 (4) for BD 1063 10 mg/kg + PRE-084 10 mg/kg, BD 1008 10 mg/kg + PRE-084 10 mg/kg, and BD 1008 30 mg/kg + PRE-084 10 mg/kg, respectively. See figure 1 legend for other details.

Figure 7.

Effects of pretreatment with BD 1063, 10 mg/kg ip (panel A), a preferential σ_1 -receptor antagonist, and BD 1008, 10 mg/kg ip (panel B), a non-selective $\sigma_{1/2}$ -receptor antagonist, on cocaine-induced stimulation of DA levels in dialysates from the NAc shell in rats. Both compounds were administered 30 min before the iv injection of a 0.32 mg/kg dose of cocaine. Basal DA values (fmol/sample) and group size (n) were: 39.1 ± 4.0 (5), and 39.2 ± 5.8 (6) for BD 1063 10 mg/kg + cocaine 0.32 mg/kg, and BD 1008 10 mg/kg + cocaine 0.32 mg/kg, respectively. See figure 1 legend for other details.

Figure 8.

Effects of removal of calcium from the perfusion Ringer's solution on DTG- and PRE-084-induced stimulation of DA levels in dialysates from the NAc shell in rats. Panels A and B show the time course of the different effects of administration of DTG or PRE-084, respectively, on stimulation of DA levels during perfusion of the microdialysis probes with normal Ringer's solution or with a calcium-free Ringer's solution. Microdialysis probes were perfused with calcium-free Ringer's solution from 60 min before injection (−60 min) until the end of the experiment (solid line above the X axis). Solid circles show the lack of effects of DTG or PRE-084 on DA levels under these conditions, as compared to their significant effects on DA levels obtained with perfusion of regular Ringer's solution (open circles). Basal DA values (fmol/sample) and group size (n) were: 27.7 ± 5.8 (4), and 42.3 ± 2.8 (4) for calcium-free Ringer's solution + PRE-084 10 mg/kg, and calcium-free Ringer's solution + DTG 5.6 mg/kg, respectively. See figure 1 legend for other details.

Table 1

Affinities of various σ -receptor ligands and cocaine on binding to σ_1 , σ_2 -receptor subtypes and to the DAT as labeled with [^3H](+)-pentazocine, [^3H]DTG, and [^3H]WIN 35,428, respectively.

Compound	σ_1 K_i Value (nM)	σ_2 K_i Value (nM)	σ_2/σ_1	DAT K_i Value (nM)
DTG *	57.4 (49.3 – 66.7)	21.9 (14.8 – 32.4) 3520 (257 – 48200)	0.382	93,500 (80,000 – 109,000)
PRE-084	53.2 (44.8 – 63.2)	32,100 (23,100 – 44,700)	603	19,600 (17,600 – 21,900)
BD 1008	2.13 (1.77 – 2.56)	16.6 (13.0 – 21.1) 20,500 (9,640 – 43,500)	7.79	2,510 (2,250 – 2,790)
BD 1047	3.13 (2.68 – 3.65)	47.5 (36.7 – 61.4) 55,300 (25,000 – 122,000)	15.2	3,220 (2,820 – 3,670)
BD 1063	8.81 (7.15 – 10.9)	625 (447 – 877) 53,700 (16,500 – 174,000)	70.9	8,020 (7,100 – 9,060)
Cocaine	5,190 (3,800 – 7,060)	19,300 (16,000 – 23,300)	3.72	76.6 (72.6 – 80.5)

* Values for σ_2 receptors are K_d values obtained by homologous competition experiments.