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The selective dopamine D₃ receptor antagonists SB-277011A and NGB 2904 and the putative partial D₃ receptor agonist BP-897 attenuate methamphetamine-enhanced brain stimulation reward in rats

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Abstract

Rationale—We have previously reported that selective antagonism of brain D₃ receptors by SB-277011A or NGB 2904 significantly attenuates cocaine- or nicotine-enhanced brain stimulation reward (BSR).

Objective—In the present study, we investigated whether the selective D₃ receptor antagonists SB-277011A and NGB 2904 and the putative partial D₃ agonist BP-897 similarly reduce methamphetamine (METH)-enhanced BSR.

Materials and methods—Rats were trained to respond for rewarding electrical self-stimulation of the medial forebrain bundle. To assess the degree of drug-induced changes in BSR, a rate–frequency curve shift paradigm was used to measure brain-reward threshold (θ_0).

Results—METH (0.1–0.65 mg/kg, i.p.) dose-dependently lowered (~10–50%) BSR thresholds, producing an enhancement of BSR. Pretreatment with SB-277011A (12 mg/kg, but not 24 mg/kg, i.p.) significantly attenuated METH-enhanced BSR. NGB 2904 (0.1–1.0 mg/kg, but not 10 mg/kg) also attenuated METH-enhanced BSR. SB-277011A or NGB 2904 alone, at the doses tested, had no effect on BSR. Pretreatment with BP-897 (0.1–5 mg/kg) dose-dependently attenuated METH-enhanced BSR. However, when the dose was increased to 10 mg/kg, BP-897 shifted the stimulation–response curve to the right (inhibited BSR itself) in the presence or absence of METH.

Conclusions—Selective antagonism of D₃ receptors by SB-277011A or NGB 2904 attenuates METH-enhanced BSR in rats, while the METH-enhanced BSR attenuation produced by BP-897 may involve both D₃ and non-D₃ receptors. These findings support a potential use of selective D₃ receptor antagonists for the treatment of METH addiction.

Keywords

Methamphetamine; Dopamine; D₃ receptor; Brain reward; SB-277011A; NGB 2904; BP-897

Introduction

Methamphetamine (METH) is a highly addictive psychostimulant and a major drug of abuse in many parts of the USA. There is currently no medication available to treat METH abuse. Like other drugs of abuse such as cocaine or nicotine, the rewarding effects of METH are believed to be mediated by elevating extracellular dopamine (DA) in the nucleus accumbens (NAc), predominantly by reversing DA transporters that facilitate cytoplasmic DA release (McCann and Ricaurte 2004; Riddle et al. 2006). Based on this DA hypothesis, development of new medications for the treatment of drug addiction has focused on manipulation of DA transmission or DA receptors in the reward circuitry of the brain.

Among the five DA receptor subtypes identified in the brain, the DA D₃ receptor has attracted much attention for development of anti-addiction medications in preclinical animal studies (Le Foll and Goldberg 2005; Heidbreder et al. 2005; Xi and Gardner 2007). This is based on the following facts: First, DA D₃ receptors have a unique anatomical distribution in that they are preferentially localized in the mesolimbic DA system, with the greatest densities of D₃ receptors in the NAc, islands of Calleja, and olfactory tubercle (Diaz et al. 2000; Stanwood et al. 2000; Sokoloff et al. 2006). Because the DA projections from the ventral tegmental area to the NAc–olfactory tubercle complex, which includes the islands of Calleja, have an important role in brain reward function (see review by Ikemoto 2007), this restricted neuroanatomic localization may underlie D₃ receptor involvement in drug reward and addiction (Le Foll and Goldberg 2005; Heidbreder et al. 2005). Second, D₃ receptors have the highest affinity for endogenous DA of all known receptors (Levant 1997; Sokoloff et al. 2001), suggesting a predominant role for D₃ receptors in the normal functioning of the mesolimbic DA system. Third, pharmacological studies demonstrate that DA D₃ receptors play an important role in emotion, motivation, and reinforcement, including the reinforcement produced by addictive drugs (Caine and Koob 1993; Duaux et al. 1998; Sokoloff et al. 2006). Growing evidence demonstrates that blockade of D₃ receptors by the selective D₃ receptor antagonists SB-277011A or NGB 2904 or by the putative partial D₃ receptor agonist BP-897 significantly inhibits reinstatement of drug-seeking behaviors triggered by addictive drugs, such as cocaine, nicotine, or alcohol, drug-associated cues or foot-shock stress (Vorel et al. 2002; Andreoli et al. 2003; Xi et al. 2004, 2006; Heidbreder et al. 2007; Cervo et al. 2007), and also inhibits cocaine or nicotine self-administration under progressive-ratio (PR), second-order, or high-effort/low-payoff reinforcement schedules (Di Ciano et al. 2003; Xi et al. 2005, 2006; Ross et al. 2007). These data support the potential

use of selective D₃ receptor antagonists or partial agonists in the clinical treatment of drug craving and relapse to drug-taking or drug-seeking behavior (see reviews by Le Foll and Goldberg 2005; Heidbreder et al. 2005; Xi and Gardner 2007). In contrast, the D₃ receptor antagonists SB-277011A and NGB 2904 or the partial D₃ agonist BP-897 have no significant effect on intravenous cocaine or nicotine self-administration under fixed-ratio reinforcement (Pilla et al. 1999; Vorel et al. 2002; Xi et al. 2005, 2006; Ross et al. 2007), but low doses attenuate cocaine- or nicotine-enhanced electrical brain stimulation reward (BSR; Pak et al. 2006; Xi et al. 2006; Xi and Gardner 2007). These data suggest that D₃ receptors may have a complex role in mediating acute rewarding effects produced by cocaine or nicotine, depending upon the doses of those addictive drugs and the animal models used to evaluate brain reward function.

Given that METH is one of the most commonly used psychostimulants, the present study was designed to determine whether blockade of DA D₃ receptors reduces METH's addictive properties in experimental animals. Specifically, in the present study, we observed and compared the effects of the selective D₃ receptor antagonists SB-277011A (Reavill et al. 2000) and NGB 2904 (Yuan et al. 1998), and the putative partial D₃ receptor agonist BP-897 (Pilla et al. 1999), on METH-enhanced BSR using intracranial self-stimulation, one of the most reliable animal models to evaluate brain reward function or rewarding responses to drugs of abuse (Wise 1996).

Materials and methods

Animals

Male Long–Evans rats (Charles River Laboratories, Raleigh, NC, USA), 300–325 g at time of surgery, were used. They were housed individually in a climate-controlled environment with food and water freely available with the exception of the time spent each day in the test chambers. All experiments were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the US National Institutes of Health and were carried out in compliance with applicable US federal and Maryland State laws and regulations. All experiments were also carried out in accordance with the principles of laboratory animal care stipulated in the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences 1996).

Surgery

Under 60 mg/kg sodium pentobarbital (i.p.) anesthesia, each rat was surgically implanted, using standard aseptic stereotaxic technique, with a unilateral monopolar stainless steel stimulating electrode (Plastics One, Roanoke, VA, USA) aimed at the medial forebrain bundle at the level of the lateral hypothalamus. The target implant stereotaxic coordinates were, from bregma, AP +2.5 mm, ML +1.7 and DV –8.4 mm, using the rat brain atlas of Paxinos and Watson (1998). The top of the electrode and the electrode connector (to which the wires from the brain stimulator are connected via a quick-connect electrical mini-plug) were cemented to the skull with acrylic resin cement. A wire wrapped around a jeweler's screw implanted in the skull and connected to a mini-pin in the electrical connector at the top of the electrode was used to accommodate return electrical current. Rats were given 1 week to recover fully from surgery, under daily veterinary supervision, before the start of experiments.

Apparatus

All training and testing occurred in standard operant chambers (MED Associates, Georgia, VT, USA), which each contained a retractable wall-mounted lever and a cue light immediately above the lever. The operant chambers were enclosed in ventilated, sound-

attenuating cabinets. Depression of the lever activated a stimulator programmed to deliver trains of 0.1-ms cathodal pulses, each pulse-train having 500-ms duration.

General procedure—The general procedures for electrical BSR were as reported previously (Xi et al. 2006; Pak et al. 2006). Briefly, after 7 days of recovery from surgery, rats were allowed to self-train (autoshape) to lever-press for rewarding BSR. Each press on the lever resulted in a 500-ms train of 0.1-ms rectangular cathodal pulses through the electrode in the rat's medial forebrain bundle at the anterior-ventral level of the lateral hypothalamus, followed by a 500-ms “timeout” in which further presses did not produce brain stimulation. The initial stimulation parameters were 72 Hz and 200 μ A. If the animal did not learn to lever-press, the stimulation intensity was increased daily by 50 μ A until the animal learned to press (45–60 responses/30 s) or a maximum of 800 μ A was reached. Animals that did not lever-press at 800 μ A or in which the stimulation produced unwanted effects (e.g., head or body movements or vocalization) were removed from the experiment.

Rate–frequency BSR procedure—After establishment of lever-pressing for BSR, animals were presented with a series of 16 different pulse frequencies, ranging from 141 to 25 Hz in descending order. At each pulse frequency, animals responded for two 30-s time periods (bins), after which the pulse frequency was decreased by 0.05 log units. After each 30-s bin, the lever retracted for 5 s. Throughout the experiment, animals were run for three sessions a day. The response rate for each frequency was defined as the mean number of lever responses during two 30-s bins. Because lever-pressing behavior tended to be variable during the first session (the “warm up” session), but was stable during the second and third sessions, the data from the first session were discarded, and the data from the second and third sessions were designated as the baseline session data and test session data, respectively. The BSR threshold (θ_0) was defined as the minimum frequency at which the animal responded for rewarding stimulation. The Ymax was defined as the maximal operant response (lever presses/30 s) for BSR. BSR threshold (θ_0) and Ymax were mathematically derived for each “baseline” run and each “drug” run by analyzing each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies using “best-fit” mathematical algorithms. Specifically, each rate–frequency BSR function was mathematically fitted, by iterative computer programs derived from the Gauss–Newton algorithm for nonlinear regression, to three different sigmoid curve-fitting mathematical growth models that appear to accurately fit rate–frequency BSR functions (Coulombe and Miliaressis 1987)—the Gompertz model ($Y' = ae^{-\alpha(b-cX)}$), the logistic model ($Y' = a/[1 + e^{(b-cX)}]$), and the Weibull function ($Y' = a[1 - e^{-(bX)^c}]$), where Y' is the rate of response (number of lever presses for rewarding brain stimulation per unit of time), X is the pulse frequency, and a , b , and c are parameters approximated from each empirical rate–frequency data curve (a representing the asymptotic response rate value, b relating to the intercept of the rate–frequency curve with the Y axis, and c representing the rate at which Y increments). From each curve-fitting model, a solution for θ_0 and a solution for Ymax were obtained. Thus, for each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies, three solutions for θ_0 and three solutions for Ymax were obtained. The three solutions for each parameter were averaged, to produce a mean value for each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies. The mean θ_0 and mean Ymax values were expressed as means $\pm$ SEM. Data analyses were performed on percent changes from baseline levels.

Testing the effects of METH or test compounds on BSR—Once a baseline θ_0 value was achieved (<10% variation over five continuous days), the effects of METH alone or METH with one dose of the experimental compounds on BSR were assessed. Five groups of animals were used to evaluate the effects of METH (0.1, 0.2, 0.4, 0.5, 0.65 mg/kg, i.p.,

$n=5$) alone on BSR or the effects of SB-277011A (3, 6, 12, 24 mg/kg, i.p., $n=10$), NGB 2904 (0.1, 0.3, 1.0, 5.0, 10 mg/kg, i.p., $n=10$), and BP-897 (0.1, 1.0, 3.0, 10 mg/kg, i.p., $n=9$) on METH (0.2 mg/kg)-enhanced BSR, respectively. All animals were injected, between the baseline and test BSR session, with an intraperitoneal (i.p.) injection of the vehicle (i.e., 25% 2-hydroxypropyl- β -cyclodextrin) or one of various doses of the test compound. Subsequently, METH or saline vehicle was then given 30 min after the test compound injection and test sessions then began immediately. After each test, animals received an additional 5–7 days of BSR restabilization until a new baseline θ_0 was established. The order of testing for various doses of drugs was counterbalanced according to a Latin square design. The effect of test compounds on METH-enhanced BSR was evaluated by comparing METH-induced alterations in θ_0 value in the presence or absence of each dose of drug pretreatment.

Drugs and chemicals

METH (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile physiological saline. SB-277011A (*trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide) was synthesized at MegaPharma Kft. (Budapest, Hungary). NGB 2904 (*N*-(4-[4-{2,3-dichlorophenyl}-1-piperazinyl]butyl)-3-fluorenylcarboxamide) was synthesized in the Medicinal Chemistry Section, Intramural Research Program, National Institute on Drug Abuse (Baltimore, MD). BP-897 (1-(4-(2-naphthoylamino)butyl)-4-(2-methoxyphenyl)-1A-piperazine) was purchased from Sigma Chemical (Saint Louis, MO). SB-277011A, NGB 2904, and BP-897 were dissolved in 25% 2-hydroxypropyl- β -cyclodextrin (Fluka Division of Sigma-Aldrich, St. Gallen, Switzerland).

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the effects of each D_3 compound on METH-enhanced BSR. Pre-planned Bonferroni t tests were used for individual group comparisons. The minimally acceptable statistical significance was set at a probability level of $p<0.05$ for all tests.

Results

Effects of METH on BSR

Figure 1a shows a representative rate–frequency function curve for BSR, indicating the BSR threshold (θ_0 , Hz) and the maximal work amount (Y_{max} , lever presses) produced by the animal for BSR. Figure 1b shows that 0.2 mg/kg METH produced a significant enhancement in BSR, as indicated by the leftward shift in the rate–frequency function curve, reflecting lowered BSR threshold (θ_0) values but without changes in Y_{max} . Also shown in Fig. 1b, this METH-enhanced BSR is substantially attenuated by the selective D_3 receptor antagonist SB-277011A (12 mg/kg). Figure 1c shows that systemic administration of METH (0.1–0.65 mg/kg) produced a significant and dose-dependent reduction (10–50%) in BSR threshold, i.e., an enhancement of brain reward. One-way ANOVA for the data shown in Fig. 1c revealed a statistically significant treatment main effect (Fig. 1c, $F_{5,20}=40.883$, $p<0.001$). Individual group comparisons revealed a statistically significant enhancement of BSR after 0.1 mg/kg ($t=4.66$, $p<0.001$), 0.2 mg/kg ($t=11.48$, $p<0.001$), 0.4 mg/kg ($t=9.76$, $p<0.001$), 0.5 mg/kg ($t=10.66$, $p<0.001$), and 0.65 mg/kg ($t=13.19$, $p<0.001$) METH administration. Figure 1d shows that the same doses of METH had no effect on Y_{max} for BSR ($F_{5,20}=0.26$, $p=NS$). In the subsequent experiments, we chose to observe the effects of test compounds on METH-enhanced BSR produced by 0.2 mg/kg METH because that dose of METH produced similar BSR enhancement (25–30%) as other addictive drugs against which we have tested DA D_3 receptor antagonists, including cocaine (2 mg/kg) and nicotine (0.25 mg/kg; Xi et al. 2006; Pak et al. 2006). Our previous studies have shown that the D_3 -selective antagonists

SB-277011A or NGB 2904 significantly attenuate the enhanced BSR produced by those relatively low doses, but not higher doses, of cocaine or nicotine in rats (Xi et al. 2006; Pak et al. 2006).

Effects of SB-277011A on METH-enhanced BSR

Pretreatment with SB-277011A (12 mg/kg, i.p., 30 min before METH injection) attenuated the METH-induced decrease in θ_0 values. Figure 2a shows that pretreatment with SB-277011A (at 12 mg/kg, but not at other doses) attenuated METH-enhanced BSR. One-way ANOVA with repeated measures for the data shown in Fig. 2a revealed a statistically significant treatment main effect ($F_{4,36}=3.54$, $p<0.05$). Individual group comparisons revealed a statistically significant reduction in METH-enhanced BSR after 12 mg/kg SB-277011A ($t=3.43$, $p<0.05$), but not after 3 mg/kg ($t=1.01$, $p=NS$), 6 mg/kg ($t=2.33$, $p=NS$), or 24 mg/kg SB-277011A ($t=0.89$, $p=NS$). Figure 2b shows that the same doses of SB-277011A had no significant effect on Ymax ($F_{4,36}=0.23$, $p=NS$). Figure 2c and d shows that SB-277011A alone had no significant effect on either BSR threshold ($F_{4,36}=2.00$, $p=NS$) or Ymax ($F_{4,36}=0.33$, $p=NS$) at any dose tested, in line with our previous reports (Vorel et al. 2002; Pak et al. 2006).

Effects of NGB 2904 on METH-enhanced BSR

Furthermore, we examined the effects of the D₃ receptor antagonist NGB 2904 on METH-enhanced BSR in a separate group of rats. As shown in Fig. 3a, NGB 2904 (at doses of 0.3 or 1.0 mg/kg, but not at other doses) significantly attenuated METH-enhanced BSR. One-way ANOVA with repeated measurements for the data shown in Fig. 3a revealed a statistically significant treatment main effect ($F_{5,40}=5.82$, $p<0.001$). Individual group comparisons revealed a statistically significant reduction in METH-enhanced BSR after 0.3 mg/kg ($t=3.55$, $p<0.05$) and 1 mg/kg NGB 2904 ($t=3.74$, $p<0.05$), but not after 0.1 mg/kg ($t=0.26$, $p=NS$), 5 mg/kg ($t=2.93$, $p=NS$), or 10 mg/kg NGB 2904 ($t=0.87$, $p=NS$). Figure 3b shows that NGB 2904 and METH treatment had no significant effect on Ymax level ($F_{5,40}=0.261$, $p=NS$). Figure 3c and d shows that NGB 2904 alone did not significantly affect BSR threshold ($F_{5,40}=0.526$, $p=NS$) or Ymax ($F_{5,40}=0.52$, $p=NS$) at any doses tested, in line with our previous reports (Xi et al. 2006).

Effects of BP-897 on METH-enhanced BSR

Finally, we observed the effects of BP-897, a D₃ receptor-selective partial agonist, on METH-enhanced BSR. Figure 4 shows that systemic administration of BP-897 (1–10 mg/kg, i.p.) significantly and dose-dependently inhibited METH-enhanced BSR, which is different from the U-shape dose-window effect produced by SB-277011A or NGB 2904, described above. One-way ANOVA with repeated measurements for the data shown in Fig. 4a revealed a statistically significant treatment main effect ($F_{4,32}=26.55$, $p<0.001$). Individual group comparisons revealed a statistically significant reduction in METH-enhanced BSR after 1.0 mg/kg ($t=2.68$, $p<0.05$), 3.0 mg/kg ($t=3.39$, $p<0.05$), and 10 mg/kg ($t=8.767$, $p<0.05$), but not after 0.1 mg/kg ($t=0.45$, $p=NS$) BP-897 administration. In addition, like the other compounds tested, BP-897 and METH did not significantly alter the Ymax at any dose tested ($F_{4,32}=0.71$, $p=NS$; Fig. 4b). Figure 4c shows that BP-897 alone, when the dose was increased to 10 mg/kg or in combination with METH (see Fig. 4a), significantly shifted the stimulation–response curve to the right, producing a significant *inhibition* of BSR ($t=8.63$, $p<0.05$; Fig. 4c). Figure 4d shows that BP-897, at any dose tested, had no effect on Ymax ($F_{4,32}=0.22$, $p=NS$).

Discussion

The electrical BSR model is one of the most reliable and sensitive animal models to assess brain reward function or brain reward responses to drugs of abuse (Wise 1996; Kornetsky 2004). Addictive drugs, such as METH, produce highly characteristic leftward shifts in BSR stimulation–response curves, i.e., reductions in BSR thresholds, indicating summation or synergism between the reward provided by the electrical stimulation and the drug-induced reward (Wise 1996; Bauco and Wise 1997). In the present study, we used this paradigm to test whether D₃ receptor antagonists have anti-METH therapeutic potential and/or intrinsic addictive potential by themselves.

The data demonstrate that systemic administration of the D₃ receptor antagonists SB-277011A or NGB 2904, within a relatively low-dose window, significantly inhibits METH-enhanced BSR. In addition, neither SB-277011A nor NGB 2904 alone, at any dose tested, significantly altered BSR compared to vehicle. In contrast to SB-277011A or NGB 2904, the partial D₃ receptor agonist BP-897 produces a dose-dependent inhibition of both METH-enhanced BSR and BSR itself. This inhibition of BSR or METH-enhanced BSR is unlikely due to non-specific sedative effects, locomotor inhibition, or locomotor impairment, as none of the compounds significantly altered Y_{max} levels, a parameter highly sensitive to locomotor inhibition (Edmonds and Gallistel 1974; Miliareisis and Rompré 1987; Fouriez et al. 1990). These data suggest that selective blockade of DA D₃ receptors by relatively low doses of SB-277011A or NGB 2904 significantly inhibits the acute rewarding effects of METH, while these D₃ receptor antagonists themselves appear to have no intrinsic rewarding effects. In contrast, the inhibition of METH's rewarding effects produced by BP-897 appears to be related to both D₃ receptor blockade and also non-D₃ receptor mechanisms because high doses of BP-897 shift the BSR function to the right, an effect similar to that produced by D₂ receptor antagonists (Ranaldi and Beninger 1994; Kita et al. 1999).

D₃ receptor antagonists have anti-addiction action, without intrinsic abuse potential

The present finding that the D₃ receptor antagonists SB-277011A and NGB 2904 inhibit METH-enhanced BSR is consistent with our previous findings demonstrating that both compounds similarly attenuate cocaine-, nicotine-, or heroin-enhanced BSR (Xi et al. 2006; Pak et al. 2006; Xi and Gardner 2007). It is also consistent with prior intravenous drug self-administration experiments demonstrating that SB-277011A or NGB 2904 dose-dependently inhibits cocaine or nicotine self-administration under PR reinforcement schedules (Xi et al. 2005, 2006; Ross et al. 2007) and prevents reinstatement of drug-seeking behavior induced by cocaine or nicotine, drug-related cues, or stress (Vorel et al. 2002; Xi et al. 2004, 2006; Andreoli et al. 2003; Cervo et al. 2007). SB-277011A is a highly potent and selective D₃ receptor antagonist that has 80- to 100-fold selectivity for D₃ over other DA receptors and 100-fold selectivity over 180 other receptors, enzymes, ion channels, and transporters in the central nervous system (Reavill et al. 2000; Stemp et al. 2000; Micheli and Heidbreder 2006; Heidbreder et al., unpublished data). Similarly, the novel D₃ receptor antagonist NGB 2904 also has >150-fold selectivity for primate D₃ over primate D₂ receptors, >800-fold selectivity for rat D₃ vs rat D₂ receptors, and >5,000-fold selectivity over D₁, D₄, and D₅ receptors (Yuan et al. 1998; Newman et al. 2003). These data suggest that the attenuation of METH-enhanced BSR by SB-277011A or NGB 2904 observed in the present study is most likely mediated by action on brain DA D₃ receptors in vivo. Furthermore, neither SB-277011A nor NGB 2904 had any significant effect on BSR itself, suggesting that selective D₃ receptor antagonists themselves have no intrinsic addictive potential, consistent with our previous reports (Vorel et al. 2002; Xi et al. 2006; Pak et al. 2006).

One of the most striking findings in the above BSR experiments is that both SB-277011A and NGB 2904 showed a U-shape dose-window effect against METH-enhanced BSR. The precise mechanisms underlying this “dose window” effect remain unclear. Given that DA D₃ receptors are distributed on both presynaptic and postsynaptic cells (see review by Joyce and Millan 2005; Sokoloff et al. 2006), we hypothesize that antagonism of postsynaptic D₃ receptors may block synaptic functional DA transmission, thereby producing the observed putative anti-addiction effects. In contrast, blockade of presynaptic D₃ receptors may augment DA release via a disinhibition mechanism, with the enhanced synaptic DA subsequently competing for postsynaptic D₃ binding sites and/or activating other DA receptor subtypes. This hypothesis is supported by our recent findings that SB-277011A or NGB 2904 dose-dependently enhanced cocaine-induced increases in NAc DA in rats (Dillon et al. 2007; Xi and Gardner 2007). Thus, it is likely that such an increase in cocaine-enhanced NAc DA produced by high doses of SB-277011A or NGB 2904 might then attenuate their anti-cocaine actions by competing with D₃ antagonist binding to postsynaptic D₃ receptors and/or by activating other DA receptors, and therefore, leading to the U-shape dose-window effect observed in the present study and others (Dillon et al. 2007; Xi and Gardner 2007). In addition, this DA enhancement hypothesis may also explain the ineffectiveness of SB-277011A or NGB 2904 on higher doses of cocaine- or nicotine-enhanced BSR (Pak et al. 2006; Xi et al. 2006), and the previous findings that SB-277011A and NGB 2904 selectively inhibit intravenous cocaine self-administration under PR, but not under FR, reinforcement schedules, because accumulative doses of cocaine under PR conditions are significantly lower than those under FR conditions. High doses of cocaine-enhanced NAc DA may similarly mitigate the putative therapeutic effects of D₃ antagonists on direct drug reward.

In contrast to the augmentation of cocaine-enhanced NAc DA described above, SB-277011A or NGB 2904 itself appeared to have no significant effect on basal levels of NAc DA (Xi et al., unpublished data). The mechanisms underlying such differential effects of D₃ antagonists on basal and cocaine-enhanced DA are unclear. The effect could be related to differential DA tone on presynaptic D₃ receptors in the absence or presence of cocaine, i.e., DA tone is low in the absence of cocaine, but increased after cocaine administration.

We should also note that we have previously reported that high doses of SB-277011A (24 mg/kg, i.p.) or NGB 2904 (5 mg/kg, i.p.) significantly inhibited PR cocaine self-administration and cocaine-triggered reinstatement of drug-seeking behavior (Xi et al. 2005, 2006), but failed to inhibit METH-enhanced BSR in the present study. The mechanisms underlying such differential effects of D₃ antagonism on actions produced by these two psychostimulants are unclear. Given that METH is more potent and effective than cocaine at elevating NAc extracellular DA, it is likely that such high doses of D₃ antagonists plus METH might produce a more robust increase in NAc DA than that produced by the same doses of D₃ antagonists plus cocaine. Whatever the mechanisms, a robust increase in NAc extracellular DA might attenuate D₃ antagonism-induced therapeutic actions as described above. In other words, postsynaptic D₃ receptor blockade might mediate the putative anti-addiction effects, while presynaptic D₃ receptor blockade might attenuate such therapeutic actions by elevating extracellular DA. Therefore, the U-shape dose-window effect may depend on the final balance of these opposite actions under different experimental conditions.

BP-897 has anti-addictive and other unwanted effects

BP-897 showed a different pattern of effects compared to SB-277011A or NGB 2904. Specifically, BP-897 produced a dose-dependent reduction in METH-enhanced BSR, which is strikingly different from the “dose-window” effect produced by SB-277011A and NGB 2904. Furthermore, as the dose increased, BP-897 produced a rightward shift of the rate-

frequency curve, suggesting an inhibition of brain reward function. Given that DA D₂ receptor antagonists have been shown to produce dysphorigenic effects in humans and a similar rightward shift of the stimulation–response BSR curve in experimental animals (Singh et al. 1996; Kita et al. 1999; Baldo et al. 1999; see review by Platt et al. 2002), we propose that this rightward shift of the stimulation–response BSR curve may suggest a potential aversive-like effect of BP-897 in humans (see review by Heidbreder et al. 2005). Clearly, this aversive-like effect is unlikely mediated by blockade of D₃ receptors because the two selective D₃ receptor antagonists did not produce aversive-like effects in the BSR paradigm nor do they produce conditioned place aversion in experimental animals (Cervo et al. 2003; Pak et al. 2006).

BP-897 was initially reported to be a D₃-selective partial agonist (Pilla et al. 1999). However, growing evidence demonstrates that BP-897 may behave as a full antagonist at both DA D₂ (pK_b=8.05) and D₃ (pK_b=9.43) receptors (Wood et al. 2000; Wicke and Garcia-Ladona 2001). Given that BP-897 has a 60- to 70-fold selectivity for human D₃ vs human D₂ receptors, and similar (60- to 70-fold) selectivity over other receptors such as α_1 -, α_2 -adrenergic receptors, and 5-HT_{1A} receptors (Pilla et al. 1999; Campiani et al. 2003), it is suggested that the aversive-like effects of BP-897 observed in the present study could be mediated by blockade of D₂ receptors or actions on other receptors. This is consistent with previous studies demonstrating that haloperidol (a preferential D₂ vs D₃ receptor antagonist) and raclopride (a mixed D₂/D₃ receptor antagonist) have dysphorigenic or aversive properties in both humans and laboratory animals (Singh et al. 1996; Kita et al. 1999; Baldo et al. 1999; see review by Platt et al. 2002). This is also consistent with previous studies demonstrating that BP-897 produces an aversive-like effect, as assessed in the BSR and conditioned place preference/aversion paradigms (Duarte et al. 2003; Gyertyán and Gál 2003; Campos et al. 2004). In addition, BP-897 also produces a compensatory increase in cocaine self-administration (Gyertyán and Gál 2003), and inhibits quinpirole (a mixed D₂/D₃ receptor agonist)-induced inhibition of DA neuronal firing in the substantia nigra, similar to the D₂ receptor antagonist haloperidol (Wicke and Garcia-Ladona 2001).

In conclusion, the present study demonstrates that selective blockade of D₃ receptors by SB-277011A or NGB 2904, within a relatively low-dose range, significantly attenuates METH-enhanced BSR. This is consistent with previous studies demonstrating that SB-277011A and NGB 2904 significantly attenuate cocaine's rewarding effects, as assessed by PR cocaine self-administration, cocaine-enhanced BSR, cocaine- or heroin-induced conditioned place preference, and reinstatement of drug-seeking behavior triggered by cocaine or nicotine, cocaine-/nicotine-associated cues, or stress (see comprehensive reviews by Heidbreder et al. 2005; Xi and Gardner 2007). Given the lack of aversive-like, sedative, or locomotor impairing effects by SB-277011A or NGB 2904, the present findings support the potential use of selective D₃ receptor antagonists in the treatment of drug addiction, including addiction to METH. In contrast to the selective D₃ receptor antagonists, the partial D₃ receptor agonist BP-897 produces an aversive-like effect in addition to its anti-addictive properties, suggesting that BP-897 may have unwanted side effects similar to other D₂-preferring antagonists. This effect probably limits its potential utility as an anti-addiction medication.

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References

- Andreoli M, Tessari M, Pilla M, Valerio E, Hagan JJ, Heidbreder CA. Selective antagonism at dopamine D₃ receptors prevents nicotine-triggered relapse to nicotine-seeking behavior. *Neuropsychopharmacology*. 2003; 28:1272–1280. [PubMed: 12700694]
- Baldo BA, Jain K, Veraldi L, Koob GF, Markou A. A dopamine D₁ agonist elevates self-stimulation thresholds: comparison to other dopamine-selective drugs. *Pharmacol Biochem Behav*. 1999; 62:659–672. [PubMed: 10208371]
- Bauco P, Wise RA. Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. *J Pharmacol Exp Ther*. 1997; 283:1160–1167. [PubMed: 9399989]
- Caine SB, Koob GF. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science*. 1993; 260:1814–1816. [PubMed: 8099761]
- Campiani G, Butini S, Trotta F, Fattorusso C, Catalanotti B, Aiello F, Gemma S, Nacci V, Novellino E, Stark JA, Cagnotto A, Fumagalli E, Carnovali F, Cervo L, Mennini T. Synthesis and pharmacological evaluation of potent and highly selective D₃ receptor ligands: inhibition of cocaine-seeking behavior and the role of dopamine D₃/D₂ receptors. *J Med Chem*. 2003; 46:3822–3839. [PubMed: 12930145]
- Campos, AC.; Xi, Z-X.; Gilbert, J.; Ashby, CR., Jr; Heidbreder, CA.; Newman, AH.; Gardner, EL. Blockade of dopamine D₃ receptors by SB-277011A, NGB 2904 or BP 897 attenuates nicotine-enhanced brain stimulation reward in rat. Abstracts of the 34th Annual Meeting of the Society for Neuroscience; San Diego, CA. 2004 November 23–27; 20042004 Abstract Viewer/Itinerary Planner. Online. Society for Neuroscience, Washington, DC. Abstract 691.6
- Cervo L, Carnovali F, Stark JA, Mennini T. Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D₃ and D₂ dopamine receptors. *Neuropsychopharmacology*. 2003; 28:1150–1159. [PubMed: 12700684]
- Cervo L, Cocco A, Petrella C, Heidbreder CA. Selective antagonism at dopamine D₃ receptors attenuates cocaine-seeking behaviour in the rat. *Int J Neuropsychopharmacol*. 2007; 10:167–181. [PubMed: 16426478]
- Coulombe D, Miliaressis E. Fitting intracranial self-stimulation data with growth models. *Behav Neurosci*. 1987; 101:209–214. [PubMed: 3580122]
- Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ. Attenuation of cue-controlled cocaine-seeking by a selective D₃ dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology*. 2003; 28:329–338. [PubMed: 12589386]
- Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz J-C, Sokoloff P. Dopamine D₃ receptors expressed by all mesencephalic dopamine neurons. *J Neurosci*. 2000; 20:8677–8684. [PubMed: 11102473]
- Dillon, C.; Li, X.; Ashby, CR., Jr; Heidbreder, CA.; Gaál, J.; Xi, ZX.; Gardner, EL. The dopamine D₃ receptor antagonist SB-277011A potentiates cocaine-induced increases in extracellular dopamine in the nucleus accumbens in rats. Abstracts of the 37th Annual Meeting for Society of Neuroscience; San Diego, CA. 2007 November 3–7; 2007
- Duarte C, Lefebvre C, Chaperon F, Hamon M, Thiébot M-H. Effects of a dopamine D₃ receptor ligand, BP 897, on acquisition and expression of food-, morphine-, and cocaine-induced conditioned place preference, and food-seeking behavior in rats. *Neuropsychopharmacology*. 2003; 28:1903–1915. [PubMed: 12915863]
- Duaux E, Gorwood P, Griffon N, Bourdel M-C, Sautel F, Sokoloff P, Schwartz J-C, Ades J, Léo H, Poirier M-F. Homozygosity at the dopamine D₃ receptor gene is associated with opiate dependence. *Mol Psychiatry*. 1998; 3:333–336. [PubMed: 9702742]
- Edmonds DE, Gallistel CR. Parametric analysis of brain stimulation reward in the rat: III. Effect of performance variables on the reward summation function. *J Comp Physiol Psychol*. 1974; 87:876–883. [PubMed: 4430753]
- Fouriez G, Bielajew C, Pagotto W. Task difficulty increases thresholds of rewarding brain stimulation. *Behav Brain Res*. 1990; 37:1–7. [PubMed: 2310490]

- Gyertyán I, Gál K. Dopamine D₃ receptor ligands show place conditioning effect but do not influence cocaine-induced place preference. *Neuroreport*. 2003; 14:93–98. [PubMed: 12544838]
- Heidbreder CA, Gardner EL, Xi Z-X, Thanos PK, Mugnaini M, Hagan JJ, Ashby CR Jr. The role of central dopamine D₃ receptors in drug addiction: a review of pharmacological evidence. *Brain Res Rev*. 2005; 49:77–105. [PubMed: 15960988]
- Heidbreder CA, Andreoli M, Marcon C, Hutcheson DM, Gardner EL, Ashby CR Jr. Evidence for the role of dopamine D₃ receptors in oral operant alcohol self-administration and reinstatement of alcohol-seeking behavior in mice. *Addict Biol*. 2007; 12:35–50. [PubMed: 17407496]
- Ikemoto S. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain Res Rev*. 2007 in press.
- Joyce JN, Millan MJ. Dopamine D₃ receptor antagonists as therapeutic agents. *Drug Discov Today*. 2005; 10:917–925. [PubMed: 15993811]
- Kita K, Shiratani T, Takenouchi K, Fukuzako H, Takigawa M. Effects of D₁ and D₂ dopamine receptor antagonists on cocaine-induced self-stimulation and locomotor activity in rats. *Eur Neuropsychopharmacol*. 1999; 9:1–7. [PubMed: 10082221]
- Kornetsky C. Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. *Neurosci Biobehav Rev*. 2004; 27:777–786. [PubMed: 15019427]
- Le Foll B, Goldberg SR. Cannabinoid CB₁ receptor antagonists as promising new medications for drug dependence. *J Pharmacol Exp Ther*. 2005; 312:875–883. [PubMed: 15525797]
- Levant B. The D₃ dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev*. 1997; 49:231–252. [PubMed: 9311022]
- McCann UD, Ricaurte GA. Amphetamine neurotoxicity: accomplishments and remaining challenges. *Neurosci Biobehav Rev*. 2004; 27:821–826. [PubMed: 15019431]
- Micheli F, Heidbreder CA. Selective dopamine D₃ receptor antagonists: a review 2001–2005. *Recent Pat CNS Drug Discov*. 2006; 1:271–288. [PubMed: 18221209]
- Miliaressis E, Rompré P-P. Effects of concomitant motor reactions on the measurement of rewarding efficacy of brain stimulation. *Behav Neurosci*. 1987; 101:827–831. [PubMed: 3426797]
- National Academy of Sciences (National Research Council, Commission on Life Sciences, Institute of Laboratory Animal Resources). *Guide for the Care and Use of Laboratory Animals*. National Academy Press; Washington DC: 1996.
- Newman AH, Cao J, Bennett CJ, Robarge MJ, Freeman RA, Luedtke RR. *N*-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl, butenyl and butynyl arylcarboxamides as novel dopamine D₃ receptor antagonists. *Bioorg Med Chem Lett*. 2003; 13:2179–2183. [PubMed: 12798330]
- Pak AC, Ashby CR Jr, Heidbreder CA, Pilla M, Gilbert J, Xi Z-X, Gardner EL. The selective dopamine D₃ receptor antagonist SB-277011A reduces nicotine-enhanced brain reward and nicotine-paired environmental cue functions. *Int J Neuropsychopharmacol*. 2006; 9:585–602. [PubMed: 16942635]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. 4th edn. Academic; San Diego: 1998.
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, Sokoloff P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D₃ receptor agonist. *Nature*. 1999; 400:371–375. [PubMed: 10432116]
- Platt DM, Rowlett JK, Spealman RD. Behavioral effects of cocaine and dopaminergic strategies for preclinical medication development. *Psychopharmacology*. 2002; 163:265–282. [PubMed: 12373428]
- Ranaldi R, Beninger RJ. The effects of systemic and intracerebral injections of D₁ and D₂ agonists on brain stimulation reward. *Brain Res*. 1994; 651:283–292. [PubMed: 7922577]
- Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, Boyfield I, Branch CL, Cilia J, Coldwell MC. Pharmacological actions of a novel, high-affinity, and selective human dopamine D₃ receptor antagonist, SB-277011-A. *J Pharmacol Exp Ther*. 2000; 294:1154–1165. [PubMed: 10945872]
- Riddle EL, Fleckenstein AE, Hanson GR. Mechanisms of methamphetamine-induced dopaminergic neurotoxicity. *AAPS J*. 2006; 8:E413–E418. [PubMed: 16808044]

- Ross JT, Corrigall WA, Heidbreder CA, LeSage MG. Effects of the selective dopamine D₃ receptor antagonist SB-277011A on the reinforcing effects of nicotine as measured by a progressive-ratio schedule in rats. *Eur J Pharmacol.* 2007; 559:173–179. [PubMed: 17303116]
- Singh J, Desiraju T, Raju TR. Dose-response functions of apomorphine, SKF 38393, LY 171555, haloperidol and clonidine on the self-stimulation evoked from lateral hypothalamus and ventral tegmentum. *Indian J Physiol Pharmacol.* 1996; 40:15–22. [PubMed: 8864766]
- Sokoloff P, Le Foll B, Perachon S, Bordet R, Ridray S, Schwartz J-C. The dopamine D₃ receptor and drug addiction. *Neurotox Res.* 2001; 3:433–441. [PubMed: 14715457]
- Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, Gross C. The dopamine D₃ receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol Disord Drug Targets.* 2006; 5:25–43. [PubMed: 16613552]
- Stanwood GD, Artymyshyn RP, Kung M-P, Kung HF, Lucki I, McGonigle P. Quantitative autoradiographic mapping of rat brain dopamine D₃ binding with [¹²⁵I]7-OH-PIPAT: evidence for the presence of D₃ receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther.* 2000; 295:1223–1231. [PubMed: 11082459]
- Stemp G, Ashmeade T, Branch CL, Hadley MS, Hunter AJ, Johnson CN, Nash DJ, Thewlis KM, Vong AKK, Austin NE. Design and synthesis of *trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): a potent and selective dopamine D₃ receptor antagonist with high oral bioavailability and CNS penetration in the rat. *J Med Chem.* 2000; 43:1878–1885. [PubMed: 10794704]
- Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL. Dopamine D₃ receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci.* 2002; 22:9595–9603. [PubMed: 12417684]
- Wicke K, Garcia-Ladona J. The dopamine D₃ receptor partial agonist, BP-897, is an antagonist at human dopamine D₃ receptors and at rat somatodendritic dopamine D₃ receptors. *Eur J Pharmacol.* 2001; 424:85–90. [PubMed: 11476753]
- Wise RA. Addictive drugs and brain stimulation reward. *Annu Rev Neurosci.* 1996; 19:319–340. [PubMed: 8833446]
- Wood MD, Boyfield I, Nash DJ, Jewitt FR, Avenell KY, Riley GJ. Evidence for antagonist activity of the dopamine D₃ receptor partial agonist, BP 897, at human dopamine D₃ receptor. *Eur J Pharmacol.* 2000; 407:47–51. [PubMed: 11050289]
- Xi Z-X, Gardner EL. Pharmacological actions of NGB-2904, a selective dopamine D₃ receptor antagonist, in animal models of drug addiction. *CNS Drug Rev.* 2007; 13:1–21. [PubMed: 17461887]
- Xi Z-X, Gilbert J, Campos AC, Kline N, Ashby CR Jr, Hagan JJ, Heidbreder CA, Gardner EL. Blockade of mesolimbic dopamine D₃ receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. *Psychopharmacology.* 2004; 176:57–65. [PubMed: 15083257]
- Xi Z-X, Gilbert JG, Pak AC, Ashby CR Jr, Heidbreder CA, Gardner EL. Selective dopamine D₃ receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost-variable-payoff fixed-ratio cocaine self-administration in rats. *Eur J Neurosci.* 2005; 21:3427–3438. [PubMed: 16026480]
- Xi Z-X, Newman AH, Gilbert JG, Pak AC, Peng X-Q, Ashby CR Jr, Gitajn L, Gardner EL. The novel dopamine D₃ receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology.* 2006; 31:1393–1405. [PubMed: 16205781]
- Yuan J, Chen X, Brodbeck R, Primus R, Braun J, Wasley JWF, Thurkauf A. NGB 2904 and NGB 2849: two highly selective dopamine D₃ receptor antagonists. *Bioorg Med Chem Lett.* 1998; 8:2715–2718. [PubMed: 9873609]

Fig. 1.

a is a representative rate–frequency function curve for BSR, indicating the definitions of BSR threshold (θ_0) and Ymax. **b** shows typical effects of methamphetamine (*METH*) after vehicle or after SB-277011A on the BSR rate–frequency function curve. *METH* (0.2 g/kg) produces a significant leftward shift in the rate–frequency function, lowering BSR θ_0 values and thus enhancing brain reward. Pretreatment with SB-277011A (12 g/kg) significantly attenuated the *METH*-induced decrease in θ_0 values. **c** and **d** show the averaged % changes in BSR, as assessed by threshold (θ_0), and in Ymax after different doses (0.10–0.65 g/kg) of *METH*, indicating that *METH* dose-dependently enhanced BSR while having no significant effect on Ymax levels. *** $p < 0.001$, compared with vehicle (0 mg/kg *METH*) treatment group

Fig. 2.

Effect of the D₃ receptor antagonist SB-277011A (3–24 mg/kg, i.p.) on METH-enhanced BSR. **a** shows that SB-277011A, at 12 mg/kg, but not at other doses, significantly attenuated METH-enhanced BSR, as assessed by BSR threshold (θ_0). **b** shows that SB-277011A and METH failed to alter Ymax levels. **c** and **d** show that SB-277011A alone failed to alter either BSR itself or Ymax levels at any doses tested. * $p < 0.05$, compared with the vehicle (0 mg/kg SB-277011A) treatment group

Fig. 3.

Effect of the D₃ receptor antagonist NGB 2904 (0.1–10 mg/kg, i.p.) on METH-enhanced BSR. **a** shows that NGB 2904, at 0.3–1.0 mg/kg, but not other doses, significantly attenuated METH-enhanced BSR, as assessed by BSR threshold (θ_0). **b** shows that NGB 2904 and METH failed to alter Ymax levels. **c** shows that NGB 2904 alone failed to alter either BSR itself or Ymax levels at any doses tested. * $p < 0.05$, compared with the vehicle (0 mg/kg NGB 2904) treatment group

Fig. 4.

Effect of the partial D_3 receptor agonist BP-897 (0.1–10 mg/kg, i.p.) on METH-enhanced BSR. **a** shows that BP-897 dose-dependently attenuated METH-enhanced BSR, as assessed by BSR threshold (θ_0). **b** shows that BP-897 and METH had no effect on Ymax levels. **c** shows that BP-897 alone dose-dependently attenuated BSR itself. **d** shows that BP-897 alone failed to alter Ymax levels at any doses tested. * $p < 0.05$, compared with the vehicle (0 mg/kg BP-897) treatment group