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N-alkyl substituted analogs of the σ receptor ligand BD1008 and traditional σ receptor ligands affect cocaine-induced convulsions and lethality in mice

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Abstract

Cocaine binds to σ receptors with comparable affinity to its well-established interaction with dopamine transporters. Previous studies have shown BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine) to have high affinity and selectivity for σ receptors, and to additionally attenuate the locomotor stimulatory effects of cocaine. Therefore, in the present study, three *N*-alkyl substituted analogs of BD1008 were characterized in receptor binding and behavioral studies: BD1060 (*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamine), BD1067 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-ethyl-2-(1-pyrrolidinyl)ethylamine), and BD1052 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-allyl-2-(1-pyrrolidinyl)ethylamine). Similarly to BD1008, all three analogs exhibited high affinity and selectivity for σ receptors. In behavioral studies, BD1008, BD1060 or BD1067 attenuated cocaine-induced convulsions and lethality in Swiss Webster mice. The protective effects appear to be mediated through σ receptor antagonism because traditional σ receptor antagonists with high to moderate affinity for these receptors also attenuated the behavioral toxicity of cocaine. In contrast, traditional and novel σ receptor agonists such as di-*o*-tolylguanidine and BD1052 worsened the behavioral toxicity of cocaine. To further characterize the actions of the *N*-alkyl substituted compounds, they were microinjected into the rat red nucleus, a functional assay of σ receptor activity, where they produced agonist vs. antagonist actions that were consistent with their effects on cocaine-induced behaviors. Together, the data demonstrate that BD1008, BD1060 or BD1067 can attenuate the behavioral toxicity of cocaine, most likely through functional antagonism of σ receptors.

Keywords: Cocaine; Convulsion; Dystonia; Lethality; Red nucleus; σ Receptor; Toxicity

1. Introduction

Cocaine has been reported to be responsible for more serious intoxications and deaths than any other illicit drug (Benowitz, 1993), a problem that is exacerbated by the lack of effective pharmacotherapies for treating cocaine overdose (Johnson and Vocci, 1993). The ability of cocaine to interact with σ receptors at concentrations that are achievable in vivo (Sharkley et al., 1988; Spiehler and Reed, 1985), suggests that these sites are logical targets for drug development. Moreover, the presence of σ receptors in key organ systems that are targeted by cocaine, such as the brain and heart (Novakova et al., 1995; cf. Walker et al., 1990a), underscores the potential relevance of these sites in the actions of cocaine. Earlier efforts, however, to attenuate the toxic and locomotor stimulatory actions of cocaine by targeting σ receptors were met with mixed results (Menkel et al., 1991; Ritz and George, 1997a,b; Witkin et al., 1993).

Over the past few years, significant advances have been made in our understanding of σ receptors, and countless novel ligands have been developed, including functional antagonists (cf. De Costa and He, 1994; Matsumoto et al.,

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1995). σ Receptors are today recognized as unique binding sites with a drug selectivity pattern and anatomical distribution that are distinct from any known receptor (Itzhak, 1994; Walker et al., 1990a). The biological relevance of σ receptors is supported by evidence for endogenous ligands for these sites (Connor and Chavkin, 1991; Patterson et al., 1994; Su et al., 1986), and correlations have been demonstrated between the functional effects of drugs and their σ binding affinities (Campbell et al., 1989; Kinney et al., 1995; Matsumoto et al., 1990a; Walker et al., 1993; Wu et al., 1991). Biochemical and pharmacological studies indicate the existence of multiple σ receptor subtypes, the most well characterized being the σ_1 and σ_2 sites (Quirion et al., 1992; Walker et al., 1990a), and σ receptors have been linked to the modulation or production of intracellular second messengers such as G-proteins, cGMP, inositol phosphates, protein kinase C, and Ca^{2+} (Bowen, 1994; Hayashi et al., 2000; Hong and Werling, 1998; Joseph and Bowen, 1998; Morin-Surun et al., 1999; Rao et al., 1991; Vilner and Bowen, 2000). Recently, several laboratories have sequenced and cloned possible receptor proteins (Hanner et al., 1996; Pan et al., 1998; Prasad et al., 1998; Seth et al., 1997, 1998), and antisense oligodeoxynucleotides for these sequences attenuate various functional effects (King et al., 1997; Kitaichi et al., 1997; Maurice et al., 1997).

We have previously reported that the σ receptor ligand BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine) has high affinity and selectivity for σ receptors, and additionally attenuates the locomotor stimulatory effects of cocaine (McCracken et al., 1999a). Therefore, the purpose of the present study was to further characterize the effects of BD1008 and three of its *N*-alkyl substituted analogs: BD1060 (*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamine), BD1067 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-ethyl-2-(1-pyrrolidinyl)ethylamine), and BD1052 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-allyl-2-(1-pyrrolidinyl)ethylamine) (De Costa et al., 1992). Receptor binding studies were used to measure the affinities and selectivities of the compounds for σ receptors as compared to several non-σ binding sites. In addition, the compounds were tested for their ability to attenuate the behavioral toxicity of cocaine in Swiss Webster mice. The actions of the novel compounds were further compared to those produced by historic σ receptor agonists and antagonists: BMY 14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol), haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone), reduced haloperidol (4-(4-chlorophenyl)-α-(4-fluorophenyl)-4-hydroxy-1-piperidine butanol), and rimcazole (*cis*-9-[3-(3,-dimethyl-1-piperazinyl)propyl]-9*H*-carbazole). To further evaluate the agonist vs. antagonist actions of the novel compounds, they were also tested after microinjection into the rat red nucleus, a functional assay of σ receptor activity (Matsumoto et al., 1990a, 1995; Okumura et al., 1996). Finally, the ability of intracerebroventricular infusion of the novel ligands to attenuate the convulsive effects of cocaine was tested to evaluate the contribution of brain σ receptors in the observed effects.

2. Materials and methods

2.1. Drugs

BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine), BD1052 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-allyl-2-(1-pyrrolidinyl)ethylamine), BD1060 (*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamine), and BD1067 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-ethyl-2-(1-pyrrolidinyl)ethylamine) were synthesized as described previously (De Costa et al., 1992). The structures of these novel ligands are shown in Fig. 1. Di-*o*-tolylguanidine (DTG) was purchased from Aldrich (Milwaukee, WI, USA). Cocaine hydrochloride, haloperidol, and naloxone were obtained from Sigma (St. Louis, MO, USA). Reduced haloperidol, (−)-sulpiride and rimcazole were purchased from Research Biochemicals International (Natick, MA, USA). (+)-Pentazocine was obtained from the National Institute on Drug Abuse Chemical Synthesis Program (Bethesda, MD, USA). The radioligands were obtained from NEN Life Sciences (Boston, MA, USA).

2.2. Animals

Frozen guinea pig brains (Pel-Freeze, Rogers, AR, USA) were used for the receptor binding studies. Male, Sprague–Dawley rats (150–300 g, Harlan, Indianapolis, IN, USA or Charles River, Boston, MA, USA) were used for the receptor binding and microinjection studies. Male,

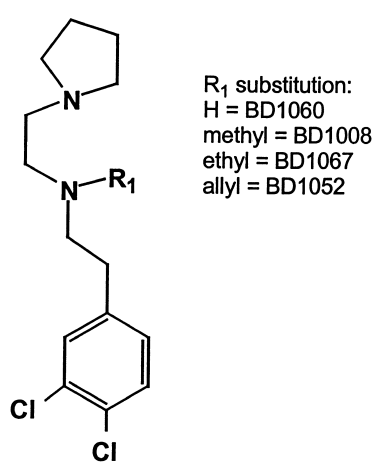

Fig. 1. Structure of BD1008 and its *N*-alkyl substituted analogs. Novel ligands and their corresponding substitutions at the R_1 position: BD1008 methyl ($-CH_3$); BD1060 H; BD1067 ethyl ($-CH_2CH_3$); BD1052 allyl ($-CH_2CH=CH_2$).

Swiss Webster mice (21–30 g, Harlan; Charles River, Portage, MI, USA) were used for behavioral testing. Before use, all animals were housed in groups with a 12:12 h light/dark cycle and ad libitum food and water. Those animals that were implanted with cannula guides were housed singly after recovering from surgery. All procedures were performed as approved by the Institutional Animal Care and Use Committee at the location where each study was conducted.

2.3. Binding affinities of novel ligands for σ and other receptors

The σ binding affinities of BD1008 and its *N*-alkyl substituted analogs were determined in tissues that are enriched in the respective subtypes using methods previously published in detail (Bowen et al., 1993; Matsumoto et al., 1995). Briefly, σ_1 sites were labeled in homogenates from guinea pig brain minus cerebellum using 3 nM [^{3}H](+)-pentazocine; σ_2 sites were labeled in homogenates from rat liver with 3 nM [^{3}H]DTG in the presence of 1 μM dextrallorphan to mask σ_1 sites. Non-specific binding was determined in the presence of 10 μM haloperidol. Twelve concentrations of test ligand (0.05–10,000 nM) were incubated for 120 min at 25°C to evaluate their ability to displace the binding of the radioligand.

Since many historic σ receptor ligands are non-specific, exhibiting interactions with dopamine, opioid, or phencyclidine (PCP) binding sites in addition to σ receptors (Tam and Cook, 1984; cf. Walker et al., 1990a), the relative selectivities of the novel ligands were determined. In addition, the affinities of the novel ligands for $GABA_A$ receptors were determined because many anticonvulsant drugs act through these receptors (Olsen et al., 1999). The affinities of the compounds for 5-HT_2 receptors were also examined because antagonists at these sites are capable of attenuating the behavioral toxicity of cocaine (Ritz and George, 1997a). The affinities of the novel ligands for dopamine, opioid, $GABA_A$, and 5-HT_2 receptors, and the PCP binding site on NMDA receptors were measured in homogenates from rat brain minus cerebellum using previously published methods (De Costa et al., 1992; Matsumoto et al., 1995). Briefly, dopamine receptors were labeled with 5 nM [^{3}H](−)sulpiride; non-specific binding was determined with 1 μM haloperidol. Opioid receptors were labeled with 2 nM [^{3}H]bremazocine; non-specific binding was determined with 10 μM levallorphan. PCP sites were labeled with 5 nM [^{3}H]TCP (1-[1-(2-thienyl)cyclohexyl]piperidine); non-specific binding was determined with 10 μM cyclazocine. $GABA_A$ receptors were labeled with 10 nM [^{3}H]muscimol; non-specific binding was determined with 1 mM GABA. 5-HT_2 receptors were labeled with 2 nM [^{3}H]ketanserin; non-specific binding was determined with 1 μM mianserin. The incubations were carried out for 60 min at 25°C for the dopamine and opioid receptor assays, 30 min at 37°C for the $GABA_A$ and 5-HT_2 receptor assays, and for 60 min at 4°C for the PCP assays.

All of the assays for σ and non-σ receptors were terminated with the addition of ice-cold buffer and vacuum filtration through glass fiber filters. Counts were extracted from the filters using Ecoscint cocktail (National Diagnostics, Manville, NJ, USA) for at least 8 h prior to counting.

2.4. Effects of intrarubral microinjection of BD1060 and BD1067 in the rat

The effects of BD1008 and BD1052 after microinjection into the rat red nucleus have already been reported (Matsumoto et al., 1999). Therefore, the actions of BD1060 and BD1067 were characterized herein using methods previously described in detail (Matsumoto et al., 1995). Briefly, chronic, indwelling cannula guides were implanted 4 mm above the red nucleus (coordinates: 2.5 mm anterior, 0.8 mm lateral, 4.0 mm ventral from lambda and the skull surface). To determine the effects of the novel ligands alone, 1 nmol/0.5 μl of BD1060 ($n = 7$) or BD1067 ($n = 9$), or an equivalent volume of saline ($n = 5$) was unilaterally microinjected into the red nucleus. The microinjections were conducted over 60 s using a motor-driven syringe pump connected to a microneedle, constructed to extend 4 mm beyond the tip of the cannula. To determine the ability of BD1060 or BD1067 (1 nmol) to attenuate σ receptor agonist-induced dystonic head postures, the compounds were coadministered intrarubrally with DTG (10 nmol; $n = 16$); the coadministrations were conducted in a total injection volume of 0.5 μl. As a control for the antagonism studies, the same animals were also coadministered DTG (1 nmol) with saline. For all of these studies, the dependent measure was the peak angle of deviation of the head from the horizontal plane during the 30-min testing session. The head angles were measured using a device that is a variation of a standard protractor. The protractor portion of the device is placed along the same horizontal plane as the animal (the placement is facilitated by using a built-in leveling feature), and a swiveling arm connected to the protractor is rotated until it is aligned through both eyes of the animal. In earlier studies, there was no significant difference between the angles obtained in real time and those of the same animals when they were photographed for these measurements. Subsequent to behavioral testing, all of the microinjection sites were histologically confirmed and only those animals with injections into the red nucleus are reported in this paper.

2.5. Effects of systemic administration of σ receptor ligands on cocaine-induced convulsions

Convulsions were induced in mice with cocaine (60 mg/kg, i.p.). Convulsions were operationally defined as

Table 1
Affinities of novel ligands for σ receptors and other binding sites

	BD1008	BD1060	BD1067	BD1052
σ Receptors				
σ_1	2 ± 1[a]	3 ± 0.1	2 ± 0.5	2 ± 0.5
σ_2	8 ± 2[a]	156 ± 45	39 ± 1	60 ± 3
Other binding sites				
Dopamine (D_2)	1112 ± 74[a]	$>10{,}000$	$>10{,}000$	$>10{,}000$
Opioid	$>10{,}000$[a]	$>10{,}000$	$>10{,}000$	$>10{,}000$
PCP	$>10{,}000$[a]	$>10{,}000$	$>10{,}000$	$>10{,}000$
$GABA_A$	$>100{,}000$	$>100{,}000$	$>100{,}000$	$>100{,}000$
5-HT_2	$>10{,}000$[a]	$>10{,}000$	$>10{,}000$	$>10{,}000$

Affinities (K_i in nM) were determined in competition assays, as described in Section 2. The values in the table represent the mean ± S.E.M. from 2–3 experiments, each performed in duplicate. Values of >10,000 nM in the table signify that there was less than 30% displacement of radioligand at this concentration.

[a] Data from McCracken et al. (1999a).

clonic or tonic limb movements, which were accompanied by the loss of righting reflexes, wild running, and/or popcorn jumping (Matsumoto et al., 1997a; Ritz and George, 1997a,b; Witkin et al., 1993). Previous studies in this laboratory have confirmed that this dose of cocaine

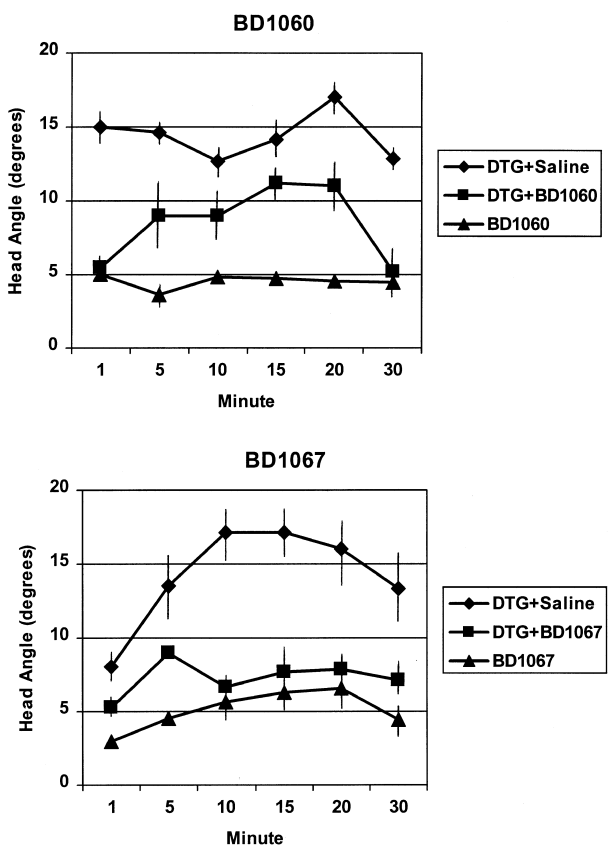

Fig. 2. Effects of BD1060 and BD1067 after microinjection into the rat red nucleus. The σ receptor agonist DTG (10 nmol) produced dystonia after unilateral microinjection into the rat red nucleus (DTG + Saline). Coadministration of 1 nmol of either BD1060 (DTG + BD1060, upper panel) or BD1067 (DTG + BD1067, lower panel) together with DTG (10 nmol) significantly attenuated the dystonia produced by this selective σ receptor agonist. As expected of an antagonist, unilateral microinjection of BD1060 or BD1067 (1 nmol/0.5 μl) alone into the rat red nucleus produced no significant effects on head posture.

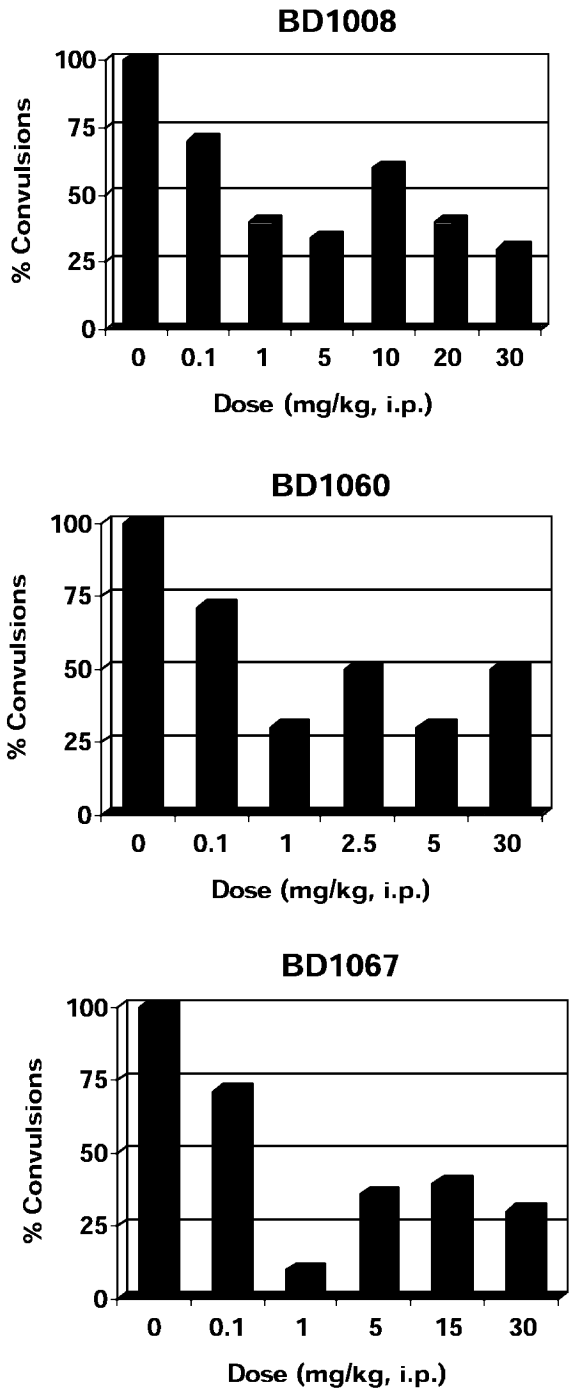

Fig. 3. Effects of BD1008, BD1060 and BD1067 on cocaine-induced convulsions. Mice were pre-treated with one of the compounds (0–30 mg/kg, i.p.), followed 15 min later with a convulsive dose of cocaine (60 mg/kg, i.p.). The data are represented as the number of mice convulsing during the 30-min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's exact tests, $P < 0.05$).

produces convulsions in 100% of our mice (Brackett et al., 2000; Matsumoto et al., 1997a; McCracken et al., 1999b). Due to the steepness of the dose–response curve for cocaine-induced convulsions (Brackett et al., 2000), and the limited supply of some of the novel compounds, only a single challenge dose of cocaine was used in these studies.

Mice were injected (i.p.) 15 min before administration of a convulsive dose of cocaine (60 mg/kg, i.p.) with one of the following pre-treatments: BD1008 (0.1, 1, 5, 10, 20, 30 mg/kg, $n = 58$), BD1060 (0.1, 2.5, 5, 15, 30 mg/kg, $n = 42$), BD1067 (0.1, 1, 5, 15, 30 mg/kg, $n = 48$), haloperidol (0.1, 1, 5, 10, 30 mg/kg, $n = 39$), reduced haloperidol (0.1, 1, 5, 10, 30 mg/kg, $n = 39$), BMY 14802 (0.1, 1, 5, 15, 30 mg/kg, $n = 40$), naloxone (1, 10, 30 mg/kg, $n = 22$), rimcazole (1, 15, 30, 60 mg/kg, $n = 24$), (−)-sulpiride (10, 30 mg/kg, $n = 10$), or saline ($n = 7$). After the injection with cocaine, the animals were observed for the next 30 min for the occurrence of convulsions.

To further probe the interaction of cocaine with putative agonists, mice were pre-treated (i.p.) with DTG (30 mg/kg, $n = 47$), (+)-pentazocine (30 mg/kg, $n = 26$), or BD1052 (30 mg/kg, $n = 39$), followed 15 min later with a dose of cocaine (5–60 mg/kg, i.p.) to determine whether the combination produced a shift to the left of the cocaine dose curve, indicating increased behavioral toxicity.

2.6. Effects of systemic administration of σ receptor ligands on cocaine-induced lethality

Mice were injected (i.p.) 15 min before administration of a lethal dose of cocaine (125 mg/kg, i.p.) with one of the following pre-treatments: BD1008 (1, 5, 15, 20, 30 mg/kg, $n = 45$), BD1052 (30 mg/kg, $n = 6$), BD1060 (1, 5, 10, 15 mg/kg, $n = 35$), BD1067 (0.1, 1, 5, 15, 30 mg/kg, $n = 36$), haloperidol (1, 5, 10, 30 mg/kg, $n = 30$), reduced haloperidol (1, 5, 10, 30 mg/kg, $n = 23$), BMY14802 (1, 15, 30 mg/kg, $n = 15$), rimcazole (1, 15, 30 mg/kg, $n = 13$), DTG (30 mg/kg, $n = 5$), naloxone (1, 10, 30, 50 mg/kg, $n = 16$), (+)-pentazocine (30 mg/kg, $n = 5$), or saline ($n = 9$). The mice were watched for 30 min following the cocaine injections and deaths were recorded. Similarly to the convulsion studies, due to the steepness of the dose–response curve for cocaine-induced lethality (Brackett et al., 2000), a single challenge dose of cocaine was used.

2.7. Effects of intracerebroventricular administration of σ receptor ligands on cocaine-induced convulsions

Mice were surgically implanted with chronic indwelling guide cannula through which solutions could be administered intracerebroventricularly. For these surgeries, the mice were deeply anesthetized with sodium pentobarbital (55 mg/kg, i.p.), preceeded by a preanesthetic dose of chlorpromazine (10 mg/kg, s.c.). Guide cannulae, constructed from 24-gauge stainless steel tubing, were implanted with their tips in the left lateral ventricle: 0.3 mm posterior, 0.7 mm lateral, and 2.4 mm ventral from bregma and the skull surface. Cannulae were secured to the skull surface with U-shaped wire and dental acrylic. Stainless steel stylets kept the cannulae sealed except during drug injection.

For the intracerebroventricular administrations, the cannulated mice were gently restrained by hand then administered 1 or 5 nmol/5 μl of BD1008 ($n = 21$), BD1060 ($n = 11$), BD1067 ($n = 13$) or an equivalent volume of

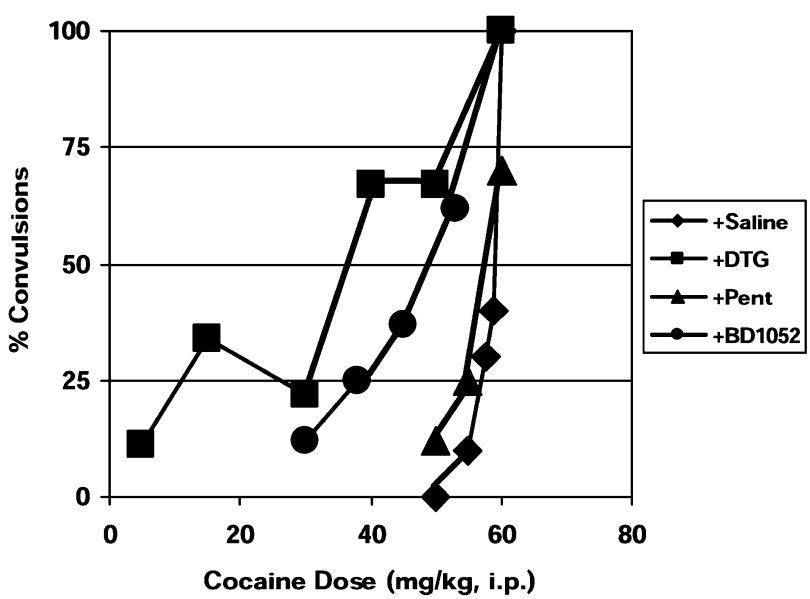

Fig. 4. Effects of σ receptor agonists on the behavioral toxicity of cocaine. Mice were pre-treated for 15 min with saline or 30 mg/kg, i.p. of DTG, BD1052, or (+)-pentazocine and subsequently administered cocaine (5–60 mg/kg, i.p.). There was a leftward shift in the dose curve for cocaine-induced convulsions in the presence of DTG or BD1052, indicating a worsening of the behavioral toxicity of cocaine.

saline ($n = 12$). After 10 min, the intracerebroventricularly injected mice were administered a normally convulsive dose of cocaine (60 mg/kg, i.p.) and observed for the next 30 min for convulsions.

Following the behavioral assessments, the cannula placements were histologically confirmed. The mice were sacrificed with an overdose of pentobarbital, cresyl violet dye (5 μl) was infused into the cannula, and the brains were removed. Coronal knife cuts were made through the site of penetration of the cannula and at the level of the cerebellum; the lateral and fourth ventricles were then examined for the presence of cresyl violet dye. Only animals with injections histologically confirmed in the ventricles are reported in this paper.

2.8. Statistics

The data from the binding assays were analyzed using GraphPad Prism (San Diego, CA, USA). The apparent K_i values of the novel ligands were calculated using the Cheng–Prusoff equation and K_d values previously determined (Bowen et al., 1993; Hellewell et al., 1994; Matsumoto et al., 1990b; unpublished observations). The data from the intrarubral microinjections were evaluated with unpaired (antagonist alone) or paired (DTG + antagonist or saline) Student's *t*-tests using the peak head angle during the 30-min testing session as the dependent measure. The data from the behavioral studies involving cocaine were analyzed with Fisher's exact tests (GraphPad InStat, San Diego, CA, USA). For all of the statistical analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Binding affinities of novel ligands for σ and non-σ receptors

The K_i values of the novel ligands for σ_1, σ_2, dopamine, opioid, PCP, $GABA_A$, and 5-HT_2 binding sites

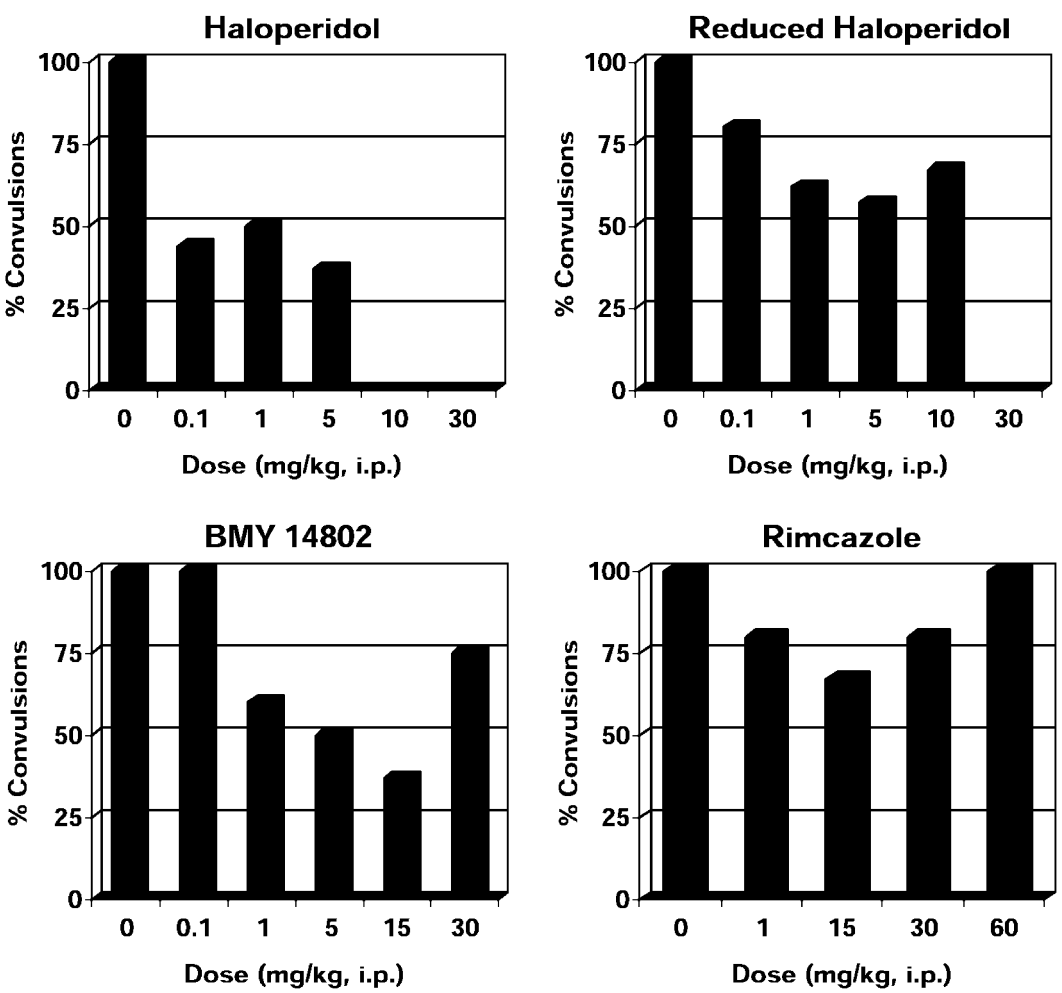

Fig. 5. Effects of traditional σ receptor ligands on cocaine-induced convulsions. Mice were pre-treated with haloperidol, reduced haloperidol, BMY14802, or rimcazole (0–60 mg/kg, i.p.), followed 15 min later with a convulsive dose of cocaine (60 mg/kg, i.p.). The data are represented as the number of mice convulsing during the 30-min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's exact tests, $P < 0.05$).

are summarized in Table 1. All of the ligands had high affinities for σ receptors. In contrast, the compounds were inactive at dopamine, opioid, PCP, $GABA_A$, and $5\text{-}HT_2$ binding sites, with the exception of BD1008 which had a low affinity for dopamine receptors (500-fold weaker than for σ receptors).

3.2. Effects of BD1060 and BD1067 after microinjection into the rat red nucleus

Student's *t*-tests revealed that when the peak head angles during the 30-min testing session were compared to those produced by a saline control, there was no significant change elicited by either BD1060 ($t = 1.21$, n.s.) or BD1067 ($t = 0.83$, n.s.) when they were unilaterally microinjected into the rat red nucleus. However, coadministration of concentrations of either BD1060 or BD1067 that produced no significant changes when administered alone, significantly attenuated the dystonia produced by the σ receptor agonist DTG (Fig. 2). Student's *t*-tests confirmed that this reduction in DTG-induced dystonia was significant for both BD1060 ($t = 7.91$, $P < 0.001$) and BD1067 ($t = 3.83$, $P < 0.005$) when compared to coadministration of saline.

3.3. Effects of systemic administration of σ receptor ligands on cocaine-induced convulsions

BD1008, BD1060, and BD1067 attenuated cocaine-induced convulsions in a dose-dependent manner ($P < 0.05$ at least one dose; Fig. 3). In contrast, the σ receptor agonists DTG, BD1052, and (+)-pentazocine were unable to prevent the convulsive effects of cocaine (Fig. 4). DTG, in fact, markedly worsened the toxicity of cocaine, shifting the ED_{50} for cocaine-induced convulsions from 58 mg/kg, i.p. in the absence of DTG to 33 mg/kg, i.p. in its presence. BD1052 similarly shifted the dose curve for cocaine-induced convulsions to the left; pre-treatment with BD1052 lowered the ED_{50} for cocaine-induced convulsions to 46 mg/kg, i.p. Pre-treatment with (+)-pentazocine, on the other hand, yielded an ED_{50} for cocaine-induced convulsions of 57 mg/kg, i.p. indicating that it does not significantly alter the responsiveness of the animals to the convulsive effects of cocaine.

Similarly to the novel ligands with BD1008-like protective effects, the high affinity σ receptor ligand, haloperidol, which is thought to act as an antagonist at σ_1 sites (Monnet et al., 1996), significantly attenuated cocaine-induced convulsions ($P < 0.05$). Reduced haloperidol, which also has high affinity for σ receptors, attenuated cocaine-induced convulsions ($P < 0.05$). BMY14802, a putative antagonist with moderate affinity for σ receptors, attenuated cocaine-induced convulsions ($P < 0.05$), but had a reduced maximal effect (maximal protection of 63% at the 15 mg/kg dose) when compared to haloperidol and reduced haloperidol (each achieving total 100% protection at their most effective doses). Rimcazole, another historic σ receptor "antagonist," failed to produce significant protection against cocaine-induced convulsions (n.s., Fisher's exact test), a pattern that is consistent with its low affinity

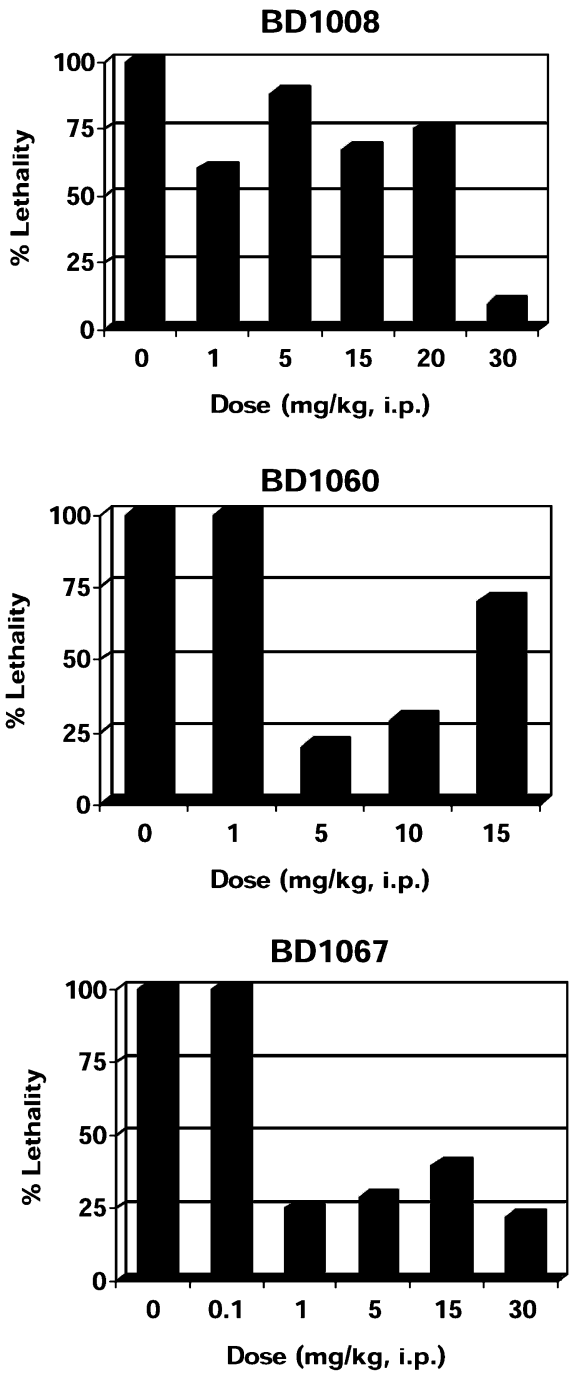

Fig. 6. Effects of BD1008, BD1060 and BD1067 on cocaine-induced lethality. Mice were pre-treated with one of the compounds, followed 15 min later with a lethal dose of cocaine (125 mg/kg, i.p.). The data are represented as the number of mice dying during the 30-min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's exact tests, $P < 0.05$).

for σ receptors. Pre-treatment with the opioid antagonist naloxone was likewise ineffective at attenuating cocaine-induced convulsions (n.s., Fisher's exact test). The dopamine D_2 receptor antagonist (−)-sulpiride was also incapable of attenuating cocaine-induced convulsions (n.s., Fisher's exact test). The effects of haloperidol, reduced haloperidol, BMY14802, and rimcazole are summarized in Fig. 5; the data from naloxone and (−)-sulpiride are not included in the figure because they were not statistically significant.

3.4. Effects of systemic administration of σ receptor ligands on cocaine-induced lethality

Pre-treatment with BD1008, BD1060, or BD1067 significantly attenuated cocaine-induced lethality ($P < 0.05$ for at least one dose; Fig. 6). Similar to the pattern observed in the convulsion studies, the σ receptor agonists DTG, BD1052, and (+)-pentazocine failed to provide significant protection against cocaine-induced lethality (n.s., Fisher's exact test). The opioid antagonist naloxone and low affinity "σ" receptor antagonist rimcazole were likewise ineffective (n.s., Fisher's exact test). In contrast, at least one tested dose of the moderate and high affinity antagonists BMY14802, haloperidol, and reduced haloperidol, significantly attenuated the lethal effects of cocaine (Fig. 7).

3.5. Effects of intracerebroventricular administration of BD1008, BD1060 or BD1067

The effects of intracerebroventricular administration of BD1008, BD1060 or BD1067 on cocaine-induced convulsions are summarized in Fig. 8. Although all of the compounds reduced the percentage of animals exhibiting cocaine-induced convulsions, Fisher's exact test revealed

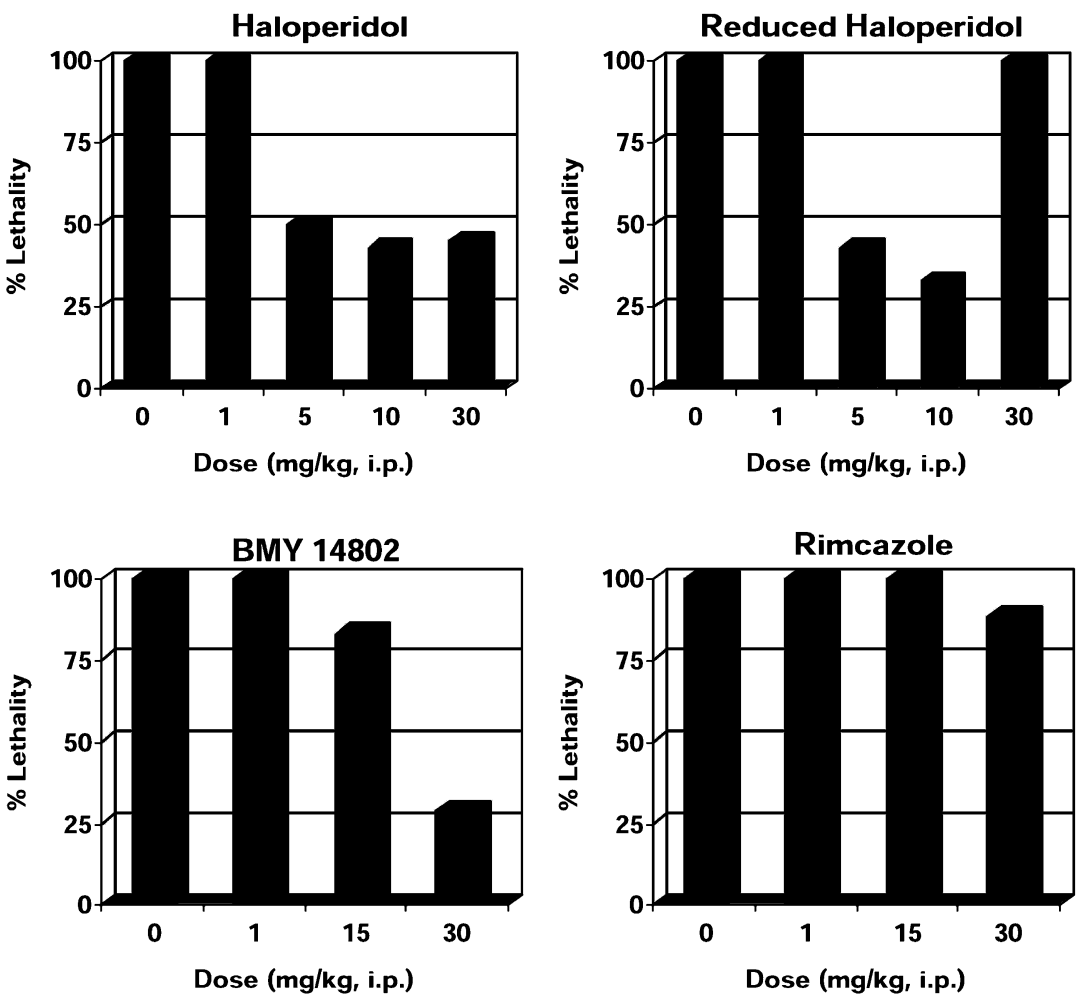

Fig. 7. Effects of traditional σ receptor ligands on cocaine-induced lethality. Mice were pre-treated with haloperidol, reduced haloperidol, BMY14802, or rimcazole (0–30 mg/kg, i.p.), followed 15 min later with a lethal dose of cocaine (125 mg/kg, i.p.). The data are represented as the number of mice dying during the 30-min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's exact tests, $P < 0.05$).

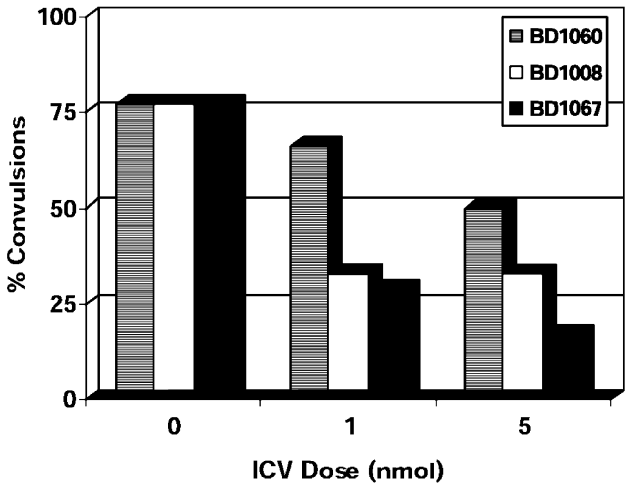

Fig. 8. Effects of intracerebroventricular administration of BD1008, BD1060 or BD1067 on cocaine-induced convulsions. Mice were injected intracerebroventricularly with BD1008, BD1060 or BD1067 (1, 5 nmol/5 μl) or an equivalent volume of saline. After 10 min, they were injected (i.p.) with a convulsive dose of cocaine (60 mg/kg, i.p.). The data are represented as the number of mice convulsing during the 30-min testing period/the total number of mice tested × 100%. The reduction produced by BD1067 was the only one that was statistically significant ($P < 0.05$).

that this difference was significant only for BD1067 ($P < 0.05$).

4. Discussion

N-alkyl substitutions to the σ receptor ligand BD1008 resulted in novel compounds with high affinity and selectivity for σ receptors. In contrast to historic σ ligands that interact with a multitude of other sites in addition to σ receptors, all of the novel σ ligands lacked significant interactions with dopamine, opioid, PCP, $GABA_A$, and 5-HT_2 sites. In addition, BD1008, the parent compound, has micromolar or lower affinities for α_1-, α_2-, β-adrenoceptors, and muscarinic and 5-HT_1 receptors (McCracken et al., 1999a). In addition to their high affinity and selectivity for σ receptors, depending on the type of *N*-alkyl substitution, the new compounds elicited either agonist or antagonist actions as determined by their effects on DTG-induced acute dystonic reactions in rats or cocaine-induced behavioral toxicity in mice.

A structure–activity analysis of the *N*-alkyl substituted analogs of BD1008 suggests that the length of the *N*-alkyl substitutions influences antagonist activity. Lengthening the substituent from H (BD1060) to methyl (BD1008) to ethyl (BD1067) appeared to improve the effectiveness of the antagonist activity without significantly changing the affinity of the compounds for the receptor. In functional studies, systemic administration of these highly selective σ receptor antagonists (BD1008, BD1060, BD1067) attenuated the convulsive and lethal effects of cocaine, with BD1067 consistently exhibiting the most favorable protection against the behavioral toxicity of cocaine. This structure activity relationship was particularly noteworthy after intracerebroventricular administration, where BD1067 (ethyl substitution) provided the greatest protection against the convulsive effects of cocaine, followed by BD1008 (methyl substitution), then BD1060. After systemic administration, the superiority of BD1067, as compared to BD1008 and BD1060, was reflected in its ability to maintain a protective effect over a wider or comparable dose range against both the convulsive and lethal endpoints. In addition to these quantitative differences, the animals treated with BD1067 appeared qualitatively more normal and similar to naïve after receiving cocaine than animals pretreated with equivalent doses of the other compounds. Therefore, among the *N*-alkyl substituents studied herein, the *N*-ethyl substitution appears to convey the best antagonist properties.

The protective actions of BD1008 and its analogs appear to be mediated through σ receptor antagonism because historic σ receptor ligands with purported antagonist actions produced a level of protection against the behavioral toxicity of cocaine that was consistent with their affinities for these receptors. For example, the classical high-affinity σ_1 receptor antagonist, haloperidol (Monnet et al., 1996), as well as its σ receptor-active metabolite reduced haloperidol (Bowen et al., 1990), significantly attenuated the convulsive effects of cocaine. BMY14802, another σ receptor-active compound with reported antagonist actions (Okumura et al., 1996), also significantly attenuated the convulsive and lethal effects of cocaine, although it was less potent than haloperidol, a pattern that is consistent with its moderate affinity for σ receptors. In contrast, rimcazole, a putative σ receptor "antagonist" with poor affinity for σ receptors (> 1400 nM; cf. Walker et al., 1990a), failed to provide significant protection against the convulsive and lethal effects of cocaine. Naloxone, an opioid receptor antagonist that is inactive at σ receptors, also failed to attenuate the convulsive and lethal effects of cocaine. Therefore, a relationship exists between the ability of historic σ receptor antagonists to produce their anti-cocaine actions and their ability to bind to the receptor.

Since all of the historic ligands interact with other binding sites in addition to σ receptors, it is noteworthy that the protective actions of the compounds cannot be explained through these non-σ receptor-mediated interactions. Particular attention was paid to potential protective effects of the historic compounds through dopamine D_2 receptors, 5-HT_{1A} receptors, or dopamine transporters because these interactions have been implicated in other physiological actions of the compounds, and could potentially impact cocaine-mediated behaviors. However, the ability of the historic compounds to attenuate cocaine-induced behavioral toxicity cannot be attributed to antagonist actions at dopamine D_2 receptors because the dopamine

D_2 receptor antagonist sulpiride did not produce similar actions. Earlier studies also suggested that of the serotonergic mechanisms, it is the 5-HT_2 receptor subtype, rather than the 5-HT_{1A}, that primarily influences cocaine-induced convulsions (Ritz and George, 1997a). Similarly, despite intensive efforts to identify compounds that block the access of cocaine to the dopamine transporter protein while still allowing normal dopamine function, all compounds from this effort have tended to possess cocaine-like effects, rather than anti-cocaine actions (cf. Appel, 2000). Thus, σ receptors are the only common site of action shared by all of the historic compounds that could explain their anti-cocaine actions.

Although the compounds in the *N*-alkyl substituted series, BD1008, BD1060 and BD1067, were capable of attenuating cocaine-induced convulsions and lethality, there were differences in the effectiveness of the ligands that could not be predicted by their binding affinities. These differences may be related to variations in the manner in which the respective ligands activate and/or occupy the receptor. In particular, earlier studies indicate that BD1008 is more appropriately designated as a partial agonist, producing functional antagonism under some conditions and weak agonist actions under others (Brent et al., 1996; Gonzalez-Alvear and Werling, 1995; Martin et al., 1994; McCracken et al., 1999a; Vilner et al., 1995). BD1060 and BD1067, on the other hand, have not been used in many functional assays to date, but the existing data suggests that BD1067 in particular, possesses a more definitive antagonist profile than BD1008.

In light of questions about the exact nature of the antagonist activities of the novel compounds, it is significant that other well-characterized and highly selective σ receptor antagonists have the ability to attenuate cocaine-induced behaviors. BD1047 (*N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-(dimethylamino)ethylamine) and BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methyl-piperazine) attenuate the convulsive, lethal, and locomotor stimulatory effects of cocaine in mice (Matsumoto et al., 1997b; McCracken et al., 1999a,b). These two compounds are the best-characterized functional antagonists at σ receptors to date, and they were first identified by their ability to attenuate DTG-induced dystonia after microinjection into the rat red nucleus (Joseph and Bowen, 1998; Matsumoto et al., 1995, 1999; Monnet et al., 1996; Tran et al., 1998). In addition to the ability of well-established pharmacological antagonists for σ receptors to prevent cocaine-induced behaviors, antisense oligodeoxynucleotides for σ receptors also attenuate the convulsive and locomotor stimulatory effects of cocaine (Matsumoto and McCracken, 1999). Therefore, interfering with cocaine's access to σ receptors using a variety of strategies to achieve functional antagonism appears to result in protective actions.

If σ receptor antagonists have anti-cocaine actions, then it is reasonable to expect that σ receptor agonists should worsen the toxicity of cocaine. Of the *N*-alkyl substituted analogs described herein, the allyl-substituted analog, BD1052, exhibited definite agonist activity across a number of systems, including the ability to elicit dystonia after microinjection into the rat red nucleus (Matsumoto et al., 1999), and a worsening of the behavioral toxicity of cocaine. This pattern was similar to that produced by the historic and selective σ receptor agonist DTG, which also produced dystonia after microinjection into the rat red nucleus and shifted the ED_{50} for cocaine-induced convulsions to the left (Matsumoto et al., 1990a; McCracken et al., 1999b; Walker et al., 1988). Since the σ receptor agonist (+)-pentazocine is also known to produce dystonia after microinjection into the rat red nucleus (Matsumoto et al., 1996), it was tested in combination with cocaine. In contrast to DTG and BD1052, (+)-pentazocine, which is often thought of as a selective σ_1 receptor agonist (Monnet et al., 1996) failed to shift the dose curve for cocaine-induced convulsions. This somewhat unexpected finding with (+)-pentazocine is thought to result from the known ability of the drug to interact with uncharacterized non-σ_1/σ_2 sites under in vivo conditions (Matsumoto et al., 1996; Walker et al., 1990b). Alternately, since (+)-pentazocine is also capable of interacting at a different position on the receptor complex from DTG in the rodent brain (Bowen et al., 1989), it is possible that DTG and BD1052 vs. (+)-pentazocine differ in their ability to impede cocaine's access to the receptor.

Two other curious features of the data that are related to deviations from traditional, expected dose–response curves also require comment. First, the dose curves for some of the protective actions against cocaine are quite steep, with more of an all-or-none effect than a traditional graded dose–response. Second, the protective effects of the antagonists against cocaine-induce lethality are often U-shaped. Both of these features may result from mechanistic properties related to cocaine itself. Imaging studies show that cocaine must occupy almost half of its receptors before its reinforcing effects are perceptible in humans, and that at a dose that is typically abused by addicts, receptor occupation is in the 80–90% range (Gatley et al., 1997). The dose–response curves for the convulsive, lethal, and locomotor stimulatory effects of cocaine in rodents are also very steep (Brackett et al., 2000; McCracken et al., 1999a,b), again suggesting that a large proportion of cocaine's receptors must be occupied for its physiological effects to be apparent in subjects. Given this pattern of activity for cocaine, it would not be surprising for a true cocaine antagonist to also have a relatively steep dose–response curve, perhaps with a breakpoint at which its therapeutic effects become noticeable. The U-shaped nature of the protection against cocaine-induced lethality may also be linked to the pathophysiology of cocaine itself. While it is possible that a yet unidentified, non-σ receptor-related feature of our novel compounds may mediate the U-shaped protective effect, it should be noted that

this pattern is also seen with other well-characterized compounds. For example, one of the few other classes of compounds that have been reported to attenuate the lethal effects of cocaine are NMDA receptor antagonists (e.g. Brackett et al., 2000). Many of these compounds produce a powerful protective effect, but again, at supramaximal doses, the protective effect is lost (Brackett et al., 2000), suggesting that a physiological balance must be achieved and maintained to prevent the lethal actions of cocaine. Thus, some of the idiosyncrasies in terms of the nature of the dose–response curves expected of σ receptor antagonists that were not observed herein may be tied in to the mechanistic properties of cocaine itself.

In sum, BD1008 provides a novel base structure from which selective σ receptor ligands can be developed. The results from this study suggest that σ receptor antagonists attenuate the behavioral toxicity of cocaine, σ receptor agonists worsen the behavioral toxicity of cocaine, and control compounds (structural analogs/historic ligands) that interact only weakly with the receptor are unable to attenuate the behavioral toxicity of cocaine. Furthermore, the pattern of results observed in interactions with cocaine parallel the observed agonist vs. antagonist actions of the compounds when administered into the rat red nucleus, an established functional assay for σ receptor activity. When the data from the cocaine and dystonia studies are considered together, BD1067, the *N*-ethyl-substituted analog of BD1008, appears to be a promising new functional antagonist at σ receptors.

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References

Appel, N.M., 2000. Status update on the search for a "dopamine-sparing cocaine antagonist". Preclinical Workshop on Cocaine Medication Discovery and Development, NIDA Division of Treatment Research and Development.

Benowitz, N.L., 1993. Clinical pharmacology and toxicology of cocaine. Pharmacol. Toxicol. 72, 3–12.

Bowen, W.D., 1994. Interaction of sigma receptors with signal transduction pathways and effects of second messengers. In: Itzhak, Y. (Ed.), Sigma Receptors. Academic Press, San Diego, pp. 139–170.

Bowen, W.D., Hellewell, S.B., McGarry, K.A., 1989. Evidence for a multi-site model of the rat brain σ receptor. Eur. J. Pharmacol. 163, 309–318.

Bowen, W.D., Moses, E.L., Tolentino, P.J., Walker, J.M., 1990. Metabolites of haloperidol display preferential activity at σ receptors compared to dopamine D-2 receptors. Eur. J. Pharmacol. 177, 111–118.

Bowen, W.D., De Costa, B.R., Hellewell, S.B., Walker, J.M., Rice, K.C., 1993. [^{3}H]-(+)-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors. Mol. Neuropharmacol. 3, 117–126.

Brackett, R.L., Pouw, B., Blyden, J.F., Nour, M., Matsumoto, R.R., 2000. Prevention of cocaine-induced convulsions and lethality in mice: effectiveness of targeting different sites on the NMDA receptor complex. Neuropharmacology 39, 407–418.

Brent, P.J., Saunders, H., Dunkley, P.R., 1996. Intrasynaptosomal free calcium levels in rat forebrain synaptosomes: modulation by sigma (σ) receptor ligands. Neurosci. Lett. 211, 138–142.

Campbell, B.G., Scherz, M.W., Keana, J.F.W., Weber, E., 1989. Sigma receptors inhibit contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation elicited by both electrical stimulation and exogenous serotonin. J. Neurosci. 9, 3380–3391.

Connor, M.A., Chavkin, C., 1991. Focal stimulation of specific pathways in the rat hippocampus causes a reduction in radioligand binding to the haloperidol-sensitive sigma receptor. Exp. Brain Res. 85, 528–536.

De Costa, B.R., He, X.-S., 1994. Structure–activity relationships and evolution of sigma receptor ligands (1976–present). In: Itzhak, Y. (Ed.), Sigma Receptors. Academic Press, San Diego, pp. 45–111.

De Costa, B.R., Radesca, L., Di Paolo, L., Bowen, W.D., 1992. Synthesis, characterization, and biological evaluation of a novel class of *N*-(arylethyl-*N*-alkyl-2-(1-pyrrolidinyl)ethylamines: structural requirements and binding affinity at the σ receptor. J. Med. Chem. 35, 38–47.

Gatley, S.J., Volkow, N.D., Gifford, A.N., Ding, Y.S., Logan, J., Wang, G.J., 1997. Model for estimating dopmaine transporter occupancy and subsequent increases in synaptic dopamine using positron emission tomography and carbon-11-labeled cocaine. Biochem. Pharmacol. 53, 43–52.

Gonzalez-Alvear, G.M., Werling, L.L., 1995. σ_1 Receptors in rat striatum regulate NMDA-stimulated [3H]dopamine release via a presynaptic mechanism. Eur. J. Pharmacol. 294, 713–719.

Hanner, M., Mobeius, F.F., Flandorfer, A., Knaus, H.-G., Striessnig, J., Kempner, E., Glossman, H., 1996. Purification, molecular cloning, and expression of the mammalian sigma$_1$-binding site. Proc. Natl. Acad. Sci. 93, 8072–8077.

Hayashi, T., Maurice, T., Su, T.P., 2000. Ca^{2+} signaling via sigma1-receptors: novel regulatory mechanism affecting intracellular Ca^{2+} concentration. J. Pharmacol. Exp. Ther. 293, 788–798.

Hellewell, S.B., Bruce, A., Feinstein, G., Orringer, J., Williams, W., Bowen, W.D., 1994. Rat liver and kidney contain high densities of sigma-1 and sigma-2 receptors. Characterization by ligand binding and photoaffinity labeling. Eur. J. Pharmacol., Mol. Pharmacol. Sect. 268, 9–18.

Hong, W., Werling, L.L., 1998. Investigation of the involvement of heteromeric guanine nucleotide-binding regulatory proteins in sigma$_1$ (σ_1) receptor signaling in rodent brain. Soc. Neurosci. Abstr. 24, 627.4.

Itzhak, Y., 1994. Sigma Receptors. Academic Press, San Diego.

Johnson, D.N., Vocci, F., 1993. Medications development at the National Institute on Drug Abuse: focus on cocaine. NIDA Res. Monogr. 135, 57–70.

Joseph, D.B., Bowen, W.D., 1998. Sigma receptor ligands robustly stimulate [^{35}S]GTPγS binding to intact SK-N-SH neuroblastoma cells but not SK-N-SH cell membrane preparations. Soc. Neurosci. Abstr. 24, 627.5.

King, M., Pan, Y.-X., Mei, J., Chang, A., Xu, J., Pasternak, G.W., 1997. Enhanced κ-opioid receptor-mediated analgesia by antisense targeting the σ_1 receptor. Eur. J. Pharmacol. 331, R5–R6.

Kinney, G.G., Harris, E.W., Ray, R., Hudzik, T.J., 1995. σ_2 Site-mediated inhibition of electrically-evoked guinea pig ileum longitudinal muscle/myenteric plexus contractions. Eur. J. Pharmacol. 294, 547–553.

Kitaichi, K., Chabot, J.-G., Dumont, Y., Bouchard, P., Quirion, R., 1997. Antisense oligodeoxynucleotide against the sigma$_1$ receptor regulates MK-801-induced memory deficits in mice. Soc. Neurosci. Abstr. 23, 695.23.

Martin, W.J., De Costa, B.R., Walker, J.M., 1994. Effects of σ ligands on rat cerebellar Purkinje neuron firing: an iontophoretic study. Brain Res. Bull. 35, 303–309.

Matsumoto, R.R., McCracken, K.A., 1999. Antisense oligodeoxynucleotides against σ_1 receptors reduce the convulsive and locomotor stimulatory effects of cocaine in mice. Soc. Neurosci. Abstr. 25, 123.2.

Matsumoto, R.R., Hemstreet, M.K., Lai, N.L., Thurkauf, A., De Costa, B.R., Rice, K.C., Hellewell, S.B., Bowen, W.D., Walker, J.M., 1990a. Drug specificity of pharmacological dystonia. Pharmacol. Biochem. Behav. 36, 151–155.

Matsumoto, R.R., Bowen, W.D., Walker, J.M., 1990b. Down-regulation of sigma receptors by chronic haloperidol. Prog. Clin. Biol. Res. 328, 125–128.

Matsumoto, R.R., Bowen, W.D., Tom, M.A., Vo, V.N., Truong, D.D., De Costa, B.R., 1995. Characterization of two novel σ receptor ligands: antidystonic effects in rats suggest σ receptor antagonism. Eur. J. Pharmacol. 280, 301–310.

Matsumoto, R.R., Bowen, W.D., Walker, J.M., Patrick, S.L., Zambon, A.C., Vo, V.N., Truong, D.D., De Costa, B.R., Rice, K.C., 1996. Dissociation of the motor effects of (+)-pentazocine from binding to σ_1 sites. Eur. J. Pharmacol. 301, 31–40.

Matsumoto, R.R., Brackett, R.L., Kanthasamy, A.G., 1997a. Novel NMDA/glycine site antagonists attenuate cocaine-induced behavioral toxicity. Eur. J. Pharmacol. 338, 233–242.

Matsumoto, R.R., Friedman, M., Blyden, J.F., Brown, M., De Costa, B.R., 1997b. Novel sigma ligands attenuate cocaine-induced toxicity and locomotor stimulation. Soc. Neurosci. Abstr. 23, 937.6.

Matsumoto, R.R., Bowen, W.D., De Costa, B.R., Houk, J.C., 1999. Relationship between modulation of the cerebellorubrospinal system in the in vitro turtle brain and changes in motor behavior in rats: effects of novel sigma ligands. Brain Res. Bull. 48, 497–508.

Maurice, T., Phan, V.-L., Urani, A., Patey, G., Privat, A., 1997. Inactivation of the σ_1 receptor by in vivo antisense oligodeoxynucleotide strategy in the mouse confirmed its implication in memory processes. Soc. Neurosci. Abstr. 23, 905.2.

McCracken, K.A., Bowen, W.D., Matsumoto, R.R., 1999a. Novel σ receptor ligands attenuate the locomotor stimulatory effects of cocaine. Eur. J. Pharmacol. 365, 35–38.

McCracken, K.A., Bowen, W.D., De Costa, B.R., Matsumoto, R.R., 1999b. Two novel σ receptor ligands, BD1047 and LR172, attenuate cocaine-induced toxicity and locomotor activity. Eur. J. Pharmacol. 370, 225–232.

Menkel, M., Terry, P., Pontecorvo, M., Katz, J.L., Witkin, J.M., 1991. Selective σ ligands block stimulant effects of cocaine. Eur. J. Pharmacol. 201, 251–252.

Monnet, F.P., De Costa, B.R., Bowen, W.D., 1996. Differentiation of σ ligand-activated receptor subtypes that modulate NMDA-evoked [^{3}H]-noradrenaline release in rat hippocampal slices. Br. J. Pharmacol. 119, 65–75.

Morin-Surun, M.P., Colllin, T., Denavit-Saubie, M., Baulieu, E.E., Monnet, F.P., 1999. Intracellular sigma1 receptor modulates phospholipase C and protein kinase C activities in the brainstem. Proc. Natl. Acad. Sci. 96, 8196–8199.

Novakova, M., Ela, C., Barg, J., Vogel, E., Hasin, Y., Eilam, Y., 1995. Ionotropic action of sigma receptor ligands in isolated cardiac myocytes from adult rats. Eur. J. Pharamacol. 286, 19–30.

Okumura, K., Ujike, H., Akiyama, K., Kuroda, S., 1996. BMY-14802 reversed the sigma receptor agonist-induced neck dystonia in rats. J. Neural Transm. 103, 1153–1161.

Olsen, R.W., DeLorey, T.M., Gordey, M., Kang, M.H., 1999. GABA receptor function and epilepsy. Adv. Neurol. 79, 499–510.

Pan, Y.X., Mei, J., Xu, J., Wan, B.L., Zuckerman, A., Pasternak, G.W., 1998. Cloning and characterization of a mouse σ_1 receptor. J. Neurochem. 70, 2279–2285.

Patterson, T., Connor, M.A., Chavkin, C., 1994. Recent evidence for endogenous substance(s) for sigma receptors. In: Itzhak, Y. (Ed.), Sigma Receptors. Academic Press, San Diego, pp. 171–189.

Prasad, P.D., Li, H.W., Fei, Y.J., Ganapathy, M.E., Fujita, T., Plumley, L.H., Yeng-Feng, T.L., Leibach, F.H., Ganapathy, V., 1998. Exon–intron structure, analysis of promoter region, and chromosomal localization of the human type 1 sigma receptor gene. J. Neurochem. 70, 443–451.

Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Rao, T.S., Mick, S.J., Cler, J.A., Emmett, M.R., Dilworth, V.M., Contreras, P.C., Gray, N.M., Wood, P.L., Iyengar, S., 1991. Effects of sigma ligands on mouse cerebellar cyclic guanosine monophosphate (cGMP) levels in vivo: further evidence for a functional modulation of *N*-methyl-D-aspartate (NMDA) receptor complex-mediated events by sigma ligands. Brain Res. 561, 43–50.

Ritz, M.C., George, F.R., 1997a. Cocaine-induced convulsions: pharmacological antagonism at serotonergic, muscarinic and sigma receptors. Psychopharmacology 129, 299–310.

Ritz, M.C., George, F.R., 1997b. Cocaine toxicity: concurrent influence of dopaminergic, muscarinic and sigma receptors in mediating cocaine-induced lethality. Psychopharmacology 129, 311–321.

Seth, P., Leibach, F.W., Ganapathy, V., 1997. Cloning and structural analysis of the cDNA and the gene encoding the murine type 1 sigma receptor. Biochem. Biophys. Res. Commun. 241, 535–540.

Seth, P., Fei, Y.J., Li, H.W., Huang, W., Leibach, F.H., Ganapathy, V., 1998. Cloning and functional characterization of a sigma receptor from rat brain. J. Neurochem. 70, 922–931.

Sharkley, J., Glen, K.A., Wolfe, S., Kuhar, M.J., 1988. Cocaine binding at σ receptors. Eur. J. Pharmacol. 149, 171–174.

Spiehler, V.R., Reed, D., 1985. Brain concentrations of cocaine and benzoylecgonine in fatal cases. J. Forensic Sci. 30, 1003–1011.

Su, T.-P., Weissman, A.D., Yeh, S.-Y., 1986. Endogenous ligands for sigma opioid receptors in the brain ("sigmaphin"): evidence from binding assays. Life Sci. 38, 2199–2210.

Tam, S.W., Cook, L., 1984. σ-Opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. Proc. Natl. Acad. Sci. 81, 5618–5621.

Tran, T.T., De Costa, B.R., Matsumoto, R.R., 1998. Microinjection of sigma ligands into cranial nerve nuclei produces vacuous chewing in rats. Psychopharmacology 137, 191–200.

Vilner, B.J., Bowen, W.D., 2000. Modulation of cellular calcium by sigma-2 receptors: release from intracellular stores in human SK-N-SH neuroblastoma cells. J. Pharmacol. Exp. Ther. 292, 900–911.

Vilner, B.J., De Costa, B.R., Bowen, W.D., 1995. Cytotoxic effects of sigma ligands: sigma receptor-mediated alterations in cellular morphology and viability. J. Neurosci. 15, 117–134.

Walker, J.M., Matsumoto, R.R., Bowen, W.D., Gans, D.L., Jones, K.D., Walker, F.O., 1988. Evidence for the role of haloperidol-sensitive σ-"opiate" receptors in the motor effects of antipsychotic drugs. Neurology 38, 961–965.

Walker, J.M., Bowen, W.D., Walker, F.O., Matsumoto, R.R., De Costa, B.R., Rice, K.C., 1990a. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402.

Walker, J.M., Patrick, S.L., Thurkauf, A., Rice, K.C., Bowen, W.D., 1990b. Inhibition of cholinergic analgesia by (+)-opiates. Prog. Clin. Biol. Res. 328, 81–84.

Walker, J.M., Bowen, W.D., Patrick, S.L., Williams, W.E., Mascarella, S.W., Bai, X., Carroll, F.I., 1993. A comparison of (−)-deoxybenzomorphans devoid of opioid activity with their dextrorotatory phenolic counterparts suggests role of σ_2 receptors in motor function. Eur. J. Pharmacol. 231, 61–68.

Witkin, J.M., Terry, P., Menkel, M., Hickey, P., Pontecorvo, M., Ferkany, J., Katz, J.L., 1993. Effects of the selective sigma receptor ligand, 6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methyflavone (NPC 16377), on behavioral toxic effects of cocaine. J. Pharmacol. Exp. Ther. 266, 473–482.

Wu, X.-Z., Bell, J.A., Spivak, C.E., London, E.D., Su, T.-P., 1991. Electrophysiological and binding studies on intact NCB-20 cells suggest presence of low affinity sigma receptors. J. Pharmacol. Exp. Ther. 257, 351–359.