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Two novel σ receptor ligands, BD1047 and LR172, attenuate cocaine-induced toxicity and locomotor activity

Kari A. McCracken [a], Wayne D. Bowen [b], Brian R. de Costa [b], Rae R. Matsumoto [a,*]

[a] *University of Oklahoma Health Sciences Center, College of Pharmacy, Department of Pharmacology and Toxicology, P.O. Box 26901, Oklahoma City, OK 73190, USA*

[b] *National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Laboratory of Medicinal Chemistry, Bethesda, MD 20892, USA*

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Abstract

The ability of cocaine to interact with σ receptors indicates that these sites may mediate the negative properties associated with cocaine use, such as toxicity and addiction. Previous studies have shown that the novel σ receptor ligand, BD1008 (*N*-[2-(3,4-dicholophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine), effectively protects against cocaine-induced convulsions and locomotor activity in mice. Therefore, BD1047 ([2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(diamino)ethylamine) and LR172 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine), two analogs of BD1008, were tested to determine if they also have anti-cocaine properties. Receptor binding assays showed that BD1047 and LR172 both have high affinities for σ receptors, but low to negligible affinities for dopamine, opioid, phencyclidine, and 5-HT_2 sites. In behavioral studies, pretreatment of mice with BD1047 or LR172 reduced the convulsions, lethality, and locomotor activity produced by cocaine. The data indicates a possible role for σ receptor ligands in the treatment of cocaine overdose and addiction.

Keywords: Cocaine; Toxicity; Convulsion; Psychomotor; Locomotor; σ Receptor

1. Introduction

Cocaine is responsible for more serious intoxications and death than any other illicit drug (Benowitz, 1993). There are currently no treatments available for cocaine overdoses and for the long-term treatment of addiction. For this reason, there is a desperate need to develop new pharmacotherapies to deal with the problems associated with cocaine use.

One approach to developing new drugs is to target the receptors that may mediate the adverse effects of cocaine. Cocaine interacts with σ receptors at concentrations that can be achieved in vivo (Spiehler and Reed, 1985; Sharkley et al., 1988). There is also evidence for a relationship between the toxic effects of cocaine and the affinity of cocaine for σ receptors (Ritz and George, 1993). Thus, the development of σ receptor ligands with anti-cocaine activity may be beneficial for treating cocaine overdose and addiction.

σ Receptors have gained heightened acceptance as unique binding sites with a specific pattern of drug selectivity and a distinctive distribution throughout the body (Walker et al., 1990; Itzhak, 1994). Endogenous ligands for these receptors appear to exist (Su et al., 1986; Connor and Chavkin, 1991; Patterson et al., 1994) and several new ligands, including functional antagonists, have been developed (Matsumoto et al., 1995; Maj et al., 1996). In the past, σ receptor ligands were quite non-selective so it was difficult to attribute their actions to binding at σ sites. The σ receptor ligands of today are much more selective due to a more complete understanding of the pharmacological and structural characteristics associated with the receptor.

BD1008 is a novel ligand with high affinity and selectivity for σ receptors (De Costa et al., 1992), and it has been reported to have anti-cocaine properties (Matsumoto et al., 1997b; McCracken et al., 1999). BD1008 is the parent compound of a group of analogs with varying structural differences and affinities for σ receptors. BD1047 and LR172 are two such analogs with alterations to the pyrrolidino ring of BD1008 (Fig. 1). These modifications were designed, in part, to evaluate their conse-

* Corresponding author. Tel.: +1-405-271-6593, ext. 47250; Fax: +1-405-271-7477; E-mail: rae-matsumoto@uokhsc.edu

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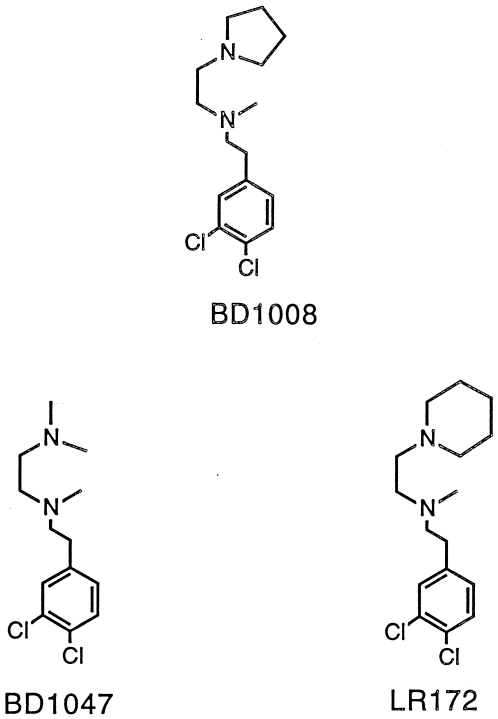

Fig. 1. Structures of BD1008, BD1047 and LR172.

quences on the antagonistic character of the compounds, and BD1047 has already been identified as a functional σ receptor antagonist in studies of its actions against the motor side effects of σ-active neuroleptics (Matsumoto et al., 1995; Tran et al., 1998). Therefore, BD1047 and LR172 were tested for their ability to attenuate cocaine-induced convulsions, lethality, and locomotor activity in mice.

2. Materials and methods

2.1. Drugs

BD1047 ([2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(diamino)ethylamine) and LR172 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine) were synthesized as previously described (De Costa et al., 1992). Cocaine hydrochloride and haloperidol were purchased from Sigma (St. Louis, MO, USA). Di-*o*-tolylguanidine (DTG) was obtained from Aldrich (Milwaukee, WI, USA). Mianserin was bought from Research Biochemicals International (Natick, MA, USA). [D-Ala^2-*N*-methyl-Phe^4, Gly-ol^5]enkephalin (DAMGO) and [D-Ser^2, Leu^5, Thr^6]enkephalin (DSTLE) were purchased from Peninsula Laboratories (Belmont, CA, USA). Levallorphan, dextrallorphan and cyclazocine were synthesized in the laboratory of Kenner C. Rice (NIDDK/NIH, Bethesda, MD, USA). The radioligands were obtained from Dupont/New England Nuclear (Boston, MA, USA) or synthesized as described previously (De Costa et al., 1989).

2.2. Animals

Male, Sprague–Dawley rats (150–200 g; Charles River, Boston, MA, USA) were used for the receptor binding studies. Frozen guinea pig brains were obtained from Pel-Freeze (Rogers, AR, USA). Male, Swiss Webster mice (21–30 g; Harlan, Indianapolis, IN, USA) were used for the behavioral experiments. Before use, all animals were housed in groups with a 12:12 light:dark cycle and ad libitum food and water. All procedures were performed as approved by the Institutional Animal Care and Use Committees at the location where each experiment took place.

2.3. Competition binding assays

The affinities of the ligands for σ receptors were determined using methods previously described in detail (Bowen et al., 1993; Matsumoto et al., 1995). Briefly, σ_1 sites were labeled in homogenates from guinea pig brains minus cerebellum using 3 nM [^{3}H](+)-pentazocine. σ_2 sites were labeled in homogenates from rat livers with 3 nM [^{3}H]DTG in the presence of 1 μM dextrallorphan to mask the σ_1 sites.

Since many historic σ receptor ligands are non-specific, and they exhibit interactions with dopamine, κ-opioid, or phencyclidine (PCP) sites in addition to σ receptors (cf. Walker et al., 1990; Itzhak, 1994), the relative selectivities of the novel σ ligands were determined. The affinities for BD1047 and LR172 for dopamine, κ-opioid, and PCP sites were measured in homogenates from rat brains minus the 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 in the presence of 1 μM haloperidol. κ-Opioid receptors were labeled with 2 nM [^{3}H]bremazocine in the presence of saturating concentrations of cold DAMGO and DSTLE to mask μ and δ receptors; non-specific binding was determined with 10 μM levallorphan. PCP sites were labeled with 5 nM [^{3}H]1-[1-(2-thienyl)cyclohexyl]piperidine hydrochloride (TCP); non-specific binding was determined with 10 μM cyclazocine. In addition, the affinities of the novel ligands for 5-HT$_2$ receptors were determined because previous studies have shown that 5-HT$_2$ antagonists can attenuate the behavioral effects of cocaine (Ritz and George, 1997). Therefore, 5-HT$_2$ sites were labeled in homogenates from rat brains minus cerebellum with 2 nM [^{3}H]ketanserin; nonspecific binding was determined with 1 μM mianserin.

2.4. Cocaine-induced convulsions

In initial experiments, the dose–response curve for cocaine-induced convulsions was determined by injecting

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

	BD1047	LR172
σ Receptors		
σ_1	0.9 ± 0.1[a]	0.4 ± 0.09
σ_2	47 ± 0.6[a]	2 ± 0.3
Other Receptors		
Dopamine (D_2)	$>10{,}000$[a]	$>10{,}000$
Opioid	$>10{,}000$[a]	$>10{,}000$
PCP	$>10{,}000$[a]	$>10{,}000$
5-HT_2	2257 ± 1110	$>10{,}000$

Affinities (in nM) were determined in competition assays, as described in Section 2. The values in the table represent the mean ± S.E.M. from two or more experiments, each performed in duplicate. Binding affinities for σ receptor subtypes are reported as K_is, while the affinities for non-σ receptors are reported as IC_{50}s. Values of $>10{,}000$ nM signify that there were less than 30% displacement of the radioligand at this concentration.
[a]Data from Matsumoto et al. (1995).

male, Swiss Webster mice (22–30 g, $n = 41$) with various doses of cocaine (50–60 mg/kg, i.p.). The animals were continuously observed for the next 30 min for the occurrence of a convulsion. Convulsions were operationally defined as clonic or tonic limb movements that were accompanied by the loss of righting reflexes, wild running, and/or popcorn jumping (Matsumoto et al., 1997a; Ritz and George, 1997).

To evaluate the effects of the novel σ receptor ligands on cocaine-induced convulsions, mice were pretreated with BD1047 (1, 5, 10, 30, 40 mg/kg, $n = 50$), LR172 (0.1, 1, 5, 15, 30 mg/kg, $n = 50$), or saline ($n = 7$). After 15 min, the mice were administered a convulsive dose of cocaine (60 mg/kg, i.p.), then observed for the next 30 min for the occurrence of convulsions. Alone, this dose of cocaine produces convulsions in 100% of our animals, and no deaths (Matsumoto et al., 1997a). Due to the limited quantities of the novel ligands and the steepness of the dose–response curve for cocaine-induced convulsions, multiple doses of the putative antagonists were tested against this single, reliable convulsive dose of cocaine. To further evaluate whether the protection provided by the novel compounds represented the actions of antagonists, animals were pretreated with the well established σ receptor agonist, DTG (20 mg/kg, i.p.), then administered one of a number of doses of cocaine (30–60 mg/kg, i.p., $n = 36$) 15 min later.

2.5. Cocaine-induced lethality

As in the convulsion study, functional antagonism was tested against a single high dose of cocaine (125 mg/kg, i.p.) that represented the LD_{97} value of cocaine determined in previous studies in our laboratory (McCracken et al., 1998). Therefore, to evaluate the effects of BD1047 and LR172 on cocaine-induced lethality, mice were injected (i.p.) 15 min prior to the administration of a lethal dose of cocaine (125 mg/kg, i.p.) with either BD1047 (0.1, 0.5, 1 mg/kg, i.p., $n = 25$), LR172 (0.1, 1, 5 mg/kg, i.p., $n = 27$) or saline ($n = 10$). The mice were watched for 30 min following the injection of cocaine and deaths were recorded.

2.6. Cocaine-induced locomotor activity

To measure locomotor activity, mice were acclimated for 30 min to the plexiglas enclosures of an automated activity monitoring system (San Diego Instruments, San

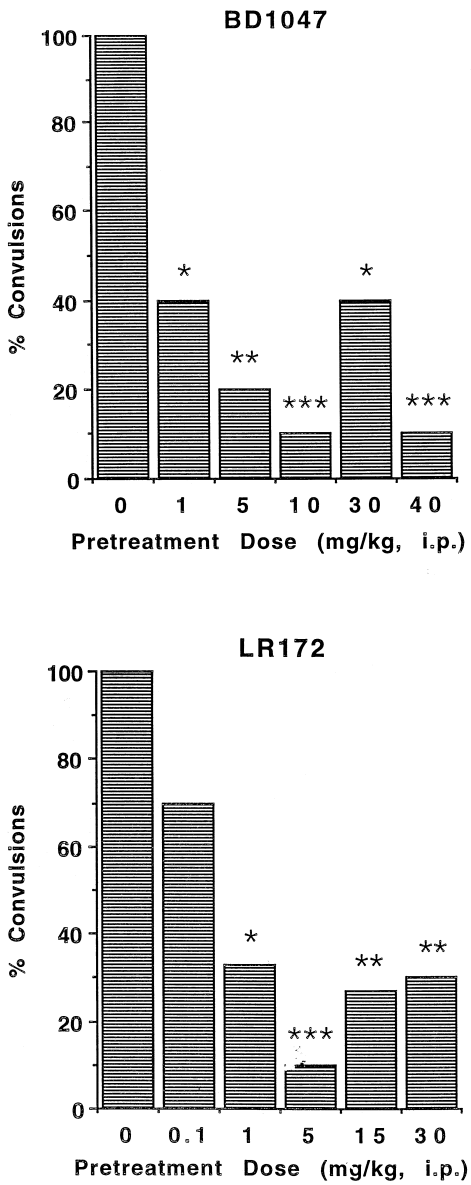

Fig. 2. BD1047 and LR172 attenuate cocaine-induced convulsions. Mice were pretreated with BD1047 (0–40 mg/kg, i.p.) or LR172 (0–30 mg/kg, i.p.), then injected 15 min later with a convulsive dose of cocaine (60 mg/kg, i.p.). Fisher's exact test revealed a significant reduction in the number of mice exhibiting cocaine-induced convulsions following pretreatment with the novel ligands. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

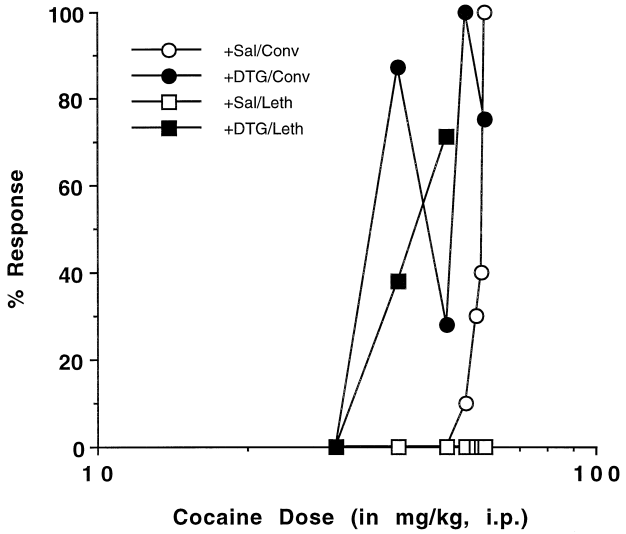

Fig. 3. DTG exacerbates the behavioral toxic effects of cocaine. Mice were pretreated with saline or the sigma receptor agonist DTG (20 mg/kg, i.p.), then injected 15 min later with varying doses of cocaine (30–60 mg/kg, i.p.). As reflected in the saline pretreatment group, these doses of cocaine elicit convulsions (+Sal/Conv), but no deaths (+Sal/Leth) at the higher doses tested in this study. Pretreatment with DTG (+DTG/Conv, +DTG/Leth) shifts the dose curve for cocaine (+Sal/Conv, +Sal/Leth) to the left.

Diego, CA, USA). After the acclimation period, horizontal locomotor activity was quantified for 30 min as the number of disruptions in the 4×4 photobeam array that surrounded each plexiglas enclosure.

In initial experiments, the dose–response curve for the locomotor stimulatory effect of cocaine was determined by injecting mice with various doses of cocaine (0–20 mg/kg, i.p., $n = 30$) after the 30 min acclimation period. Horizontal locomotor activity was quantified for the next 30 min. The dose of cocaine that produced the peak level of locomotor activity was selected for use in the subsequent antagonism portion of the study.

To select appropriate antagonist doses of the novel ligands, the effects of BD1047 and LR172 on locomotor activity were first determined. After a 30 min acclimation period, the mice were injected (i.p.) with 5 mg/kg of BD1047 ($n = 6$) or LR172 ($n = 6$) and horizontal locomotor activity was quantified for 30 min to ensure that this dose produced effects no different from saline ($n = 10$). The 5 mg/kg dose was chosen because it is a low, but effective, dose in the convulsion experiments. In later experiments, an additional 30 mg/kg (i.p.) dose of BD1047 was also tested ($n = 6$).

For the antagonism experiments, mice were acclimated to the activity monitors for 15 min. The animals were then injected (i.p.) with saline ($n = 6$) or a behaviorally inactive dose of BD1047 (5 mg/kg, $n = 6$; 30 mg/kg, $n = 6$) or LR172 (5 mg/kg, $n = 6$). After a 15 min pretreatment period, cocaine (10 mg/kg, i.p.) was administered and horizontal locomotor activity quantified for the subsequent 30 min.

2.7. Statistics

The data from the binding assays were analyzed using GraphPad Prism (San Diego, CA, USA). Apparent K_i values were calculated for the affinities of the ligands for σ receptors using the Cheng–Prusoff equation and K_d values that were previously determined (Matsumoto et al., 1990; Bowen et al., 1993; Hellewell et al., 1994). The affinities of the ligands for non-σ binding sites were calculated as IC_{50} values. The data from the convulsion studies were analyzed with Fisher's exact tests (GraphPad InStat). The data from the locomotor studies were evaluated with repeated measures analysis of variance, followed

Fig. 4. BD1047 and LR172 protect against cocaine-induced lethality. Mice were pretreated with BD1047 (0–1 mg/kg, i.p.) or LR172 (0–5 mg/kg, i.p.), then injected 15 min later with a lethal dose of cocaine (125 mg/kg, i.p.). Fisher's exact test revealed a significant reduction in the number of resulting deaths following pretreatment with the novel ligands. $^{*}P < 0.05$, $^{**}P < 0.01$.

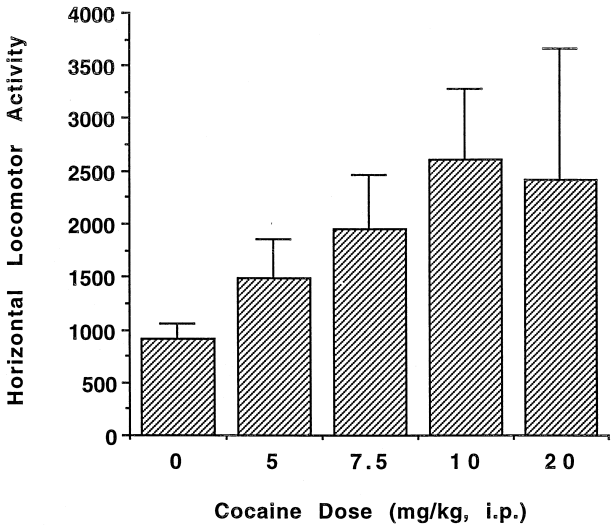

Fig. 5. The stimulatory effect of cocaine on locomotor activity at varying doses. Mice were acclimated to the test chambers for 30 min. After this time period, they were injected with a dose of cocaine (0–20 mg/kg, i.p.) and horizontal locomotor activity was measured for the following 30 min. Analysis of variance revealed a significant effect of dose, and post-hoc Dunnett's tests showed that the effects of cocaine at all of the cocaine doses differed significantly from the saline control ($P < 0.01$).

by post-hoc Dunnett's tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Binding affinities

The affinities of BD1047 and LR172 for σ_1, σ_2, dopamine, κ-opioid, PCP, and 5-HT_2 sites are indicated in Table 1. Both BD1047 and LR172 have low nanomolar affinities for σ receptors. In contrast, they have low to negligible affinities for dopamine, κ-opioid, PCP, and 5-HT_2 binding sites.

3.2. Convulsions

Pretreatment of mice with BD1047 or LR172 produced significant protection from cocaine-induced convulsions over a wide range of doses (Fig. 2). In contrast, the well-established σ receptor agonist DTG worsened the convulsive effects of cocaine, shifting the ED_{50} for cocaine-induced convulsions from 57 mg/kg (i.p.) to 46 mg/kg (i.p.). Further, within the convulsive dose range tested for cocaine (up to 60 mg/kg, i.p.), deaths were not observed (Fig. 3). Previously, we had determined that the LD_{50} for cocaine in our hands was 108 mg/kg (i.p.). However, in the presence of DTG, the LD_{50} for cocaine was reduced to 44 mg/kg (i.p.).

3.3. Lethality

Pretreatment of the mice with either BD1047 or LR172 produced significant protection against the lethal effects of cocaine (Fig. 4). In contrast, pretreatment with saline resulted in the deaths of all mice tested.

3.4. Locomotor activity

The dose–response for the locomotor stimulatory effects of cocaine is shown in Fig. 5. A repeated measures analysis of variance revealed a significant effect of cocaine dose on horizontal locomotor activity ($F[4,20] = 41.58$,

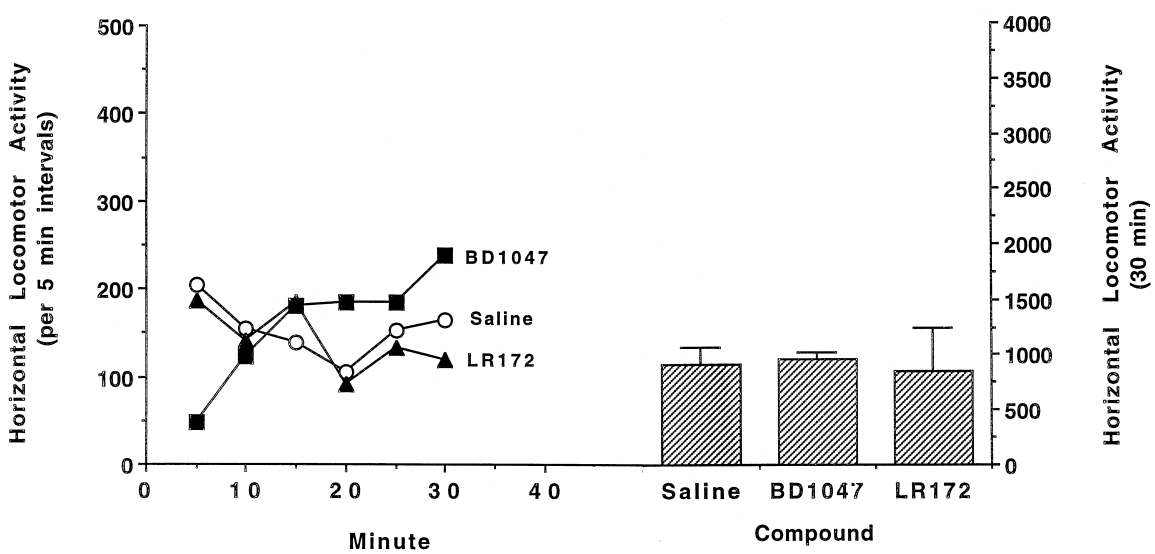

Fig. 6. Alone, BD1047 and LR172 produce effects on spontaneous locomotor activity no different from saline. Mice were acclimated to the test chambers for 30 min. After this time period, they were injected with saline, BD1047 (30 mg/kg, i.p.), or LR172 (5 mg/kg, i.p.). Horizontal locomotor activity was measured for the following 30 min.

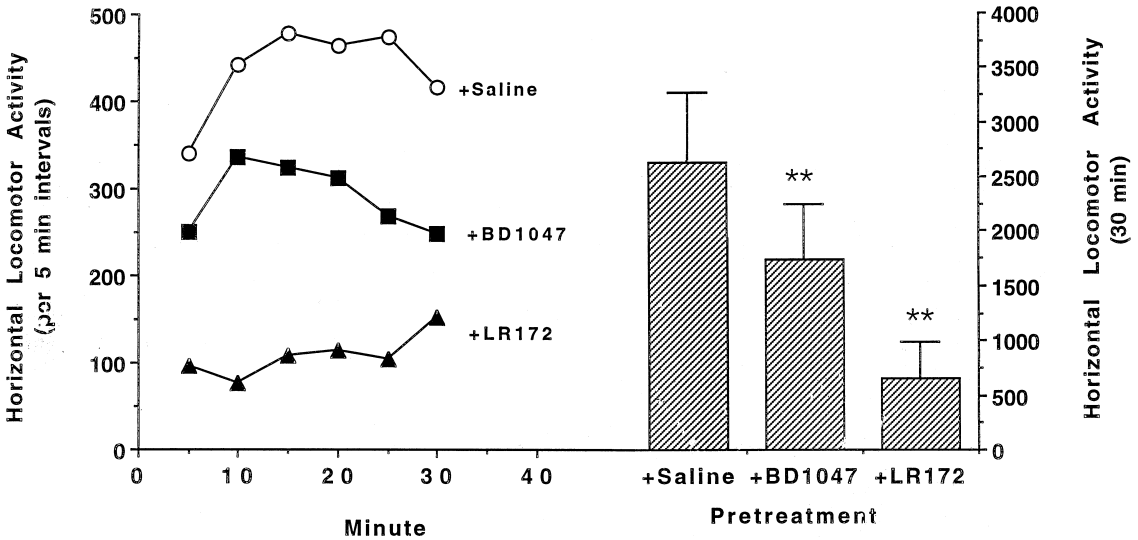

Fig. 7. BD1047 and LR172 attenuate the locomotor stimulatory effects of cocaine. Mice who were acclimated to the testing chambers were pretreated with saline, BD1047 (30 mg/kg, i.p.), or LR172 (5 mg/kg, i.p.), followed 15 min later with a locomotor stimulatory dose of cocaine (10 mg/kg, i.p.). Analysis of variance revealed a significant effect of pretreatment, and post-hoc Dunnett's tests showed that as compared to pretreatment with the saline control, BD1047 and LR172 significantly attenuated cocaine-induced locomotor activity. $^{**}P < 0.01$.

$P < 0.0001$). There was a significant difference between the saline control and each of the cocaine doses used in the dose–response curve as revealed by post-hoc Dunnett's tests (5 mg/kg: $q = 3.78$, $P < 0.01$; 7.5 mg/kg: $q = 6.87$, $P < 0.01$; 10 mg/kg: $q = 11.20$, $P < 0.01$; 20 mg/kg: $q = 9.89$, $P < 0.01$). The level of activity produced by the doses of BD1047 and LR172 that were used in the antagonism portion of the study are shown in Fig. 6. For simplicity, the data from the lower 5 mg/kg dose of BD1047 is not shown in Fig. 6, but did not differ significantly from the other groups (1399 ± 281 activity units/30 min). An analysis of variance confirmed that when administered alone, there was no significant difference between the behaviorally inactive doses of each ligand and the saline vehicle ($F[3,22] = 1.02$, n.s.). When the behaviorally inactive doses were tested against the dose of cocaine that caused the maximal locomotor stimulatory effect, BD1047 and LR172 significantly attenuated locomotor activity, as shown in Fig. 7. A repeated measures of analysis of variance confirmed a significant effect of treatment with the ligands ($F[4,20] = 135.74$, $P < 0.0001$), and post-hoc Dunnett's tests revealed a notable difference between the mice pretreated with saline vs. those pretreated with LR172 ($q = 18.11$, $P < 0.01$) or BD1047 (30 mg/kg, $q = 12.01$, $P < 0.01$; 5 mg/kg, $q = 9.49$, $P < 0.01$). Further, there appeared to be a dose effect because the higher 30 mg/kg dose of BD1047 had a greater protective effect than the lower 5 mg/kg dose.

4. Discussion

BD1047 and LR172, two structural analogs of BD1008 with alterations to the parent pyrrolidino ring, provided significant protection against cocaine-induced behavioral toxicity and locomotor activity. Both compounds have high affinities for σ receptors, and for the most part, lack significant interactions with dopamine, κ-opioid, PCP or 5-HT_2 binding sites. Although BD1047 has low micromolar affinity for 5-HT_2 receptors, and 5-HT_2 receptor antagonists can attenuate the convulsive effects of cocaine (Ritz and George, 1997), it is unlikely that these receptors are responsible for the anti-cocaine effects of BD1047 because: (1) BD1047 has a 50- to 100-fold better affinity for σ receptors vs. 5-HT_2 receptors, (2) LR172 and five other BD1008 analogs with anti-cocaine properties lack significant interactions with 5-HT_2 receptors ($IC_{50} > 10{,}000$ nM; Matsumoto et al., 1997b), and (3) EMD 57445, a novel σ receptor ligand from another synthetic series, lacks interactions with 5-HT_2 receptors, but possesses anti-cocaine actions (Maj et al., 1996). Therefore, it is likely that BD1047 and LR172 produce their protective effects through σ receptors.

The protective actions of BD1047 and LR172 appear to be related to antagonist actions of the compounds. BD1047 has previously been shown to act as a functional antagonist in other σ receptor-mediated systems (Matsumoto et al., 1995; Joseph and Bowen, 1998; Tran et al., 1998), making it likely that it would retain this activity in the studies herein. In contrast, the well established σ receptor agonist, DTG, exacerbated the toxic effects of cocaine. The pattern of results observed herein are thus consistent with data from studies involving other substituted analogs of BD1008 and σ receptor ligands in which putative antagonists prevent the toxic effect of cocaine, while agonists fail to protect or exacerbate the toxicity (Matsumoto et al., 1997b; McCracken et al., 1998). In addition, converging evidence exists from preliminary studies in which anti-cocaine ac-

tions can be elicited with a σ receptor antisense oligodeoxynucleotide, which acts as a molecular antagonist by interfering with the synthesis of σ receptors (unpublished data). Therefore, the studies indicate an agonist vs. antagonist relationship in terms of the ability of the novel ligands to worsen vs. prevent the behavioral toxic effects of cocaine.

Historically, the properties of addiction, such as reinforcement and locomotor stimulatory effects, have been attributed to dopamine receptors and the dopamine transporter (Kuhar et al., 1988). Cocaine interacts at these sites as an indirect dopamine agonist and a potent inhibitor of the dopamine transporter (Kuhar et al., 1988). Certain σ receptor ligands with high selectivity for σ receptors and low to negligible interaction with dopamine sites, such as BD1008 and now BD1047 and LR172, have been able to reduce the stimulatory effects of cocaine on locomotor activity (McCracken et al., 1999). Thus, σ receptors may play a modulatory role in alleviating the properties associated with addiction.

Although the anti-cocaine properties of these novel ligands seem to be mediated through σ receptors, it is less clear which σ receptor subtype is involved in the protection. Both compounds have high, nanomolar affinities for the σ_1 receptor subtype but have varying affinities for the σ_2 receptor subtype. Therefore, it can be hypothesized that since both of the σ_1 receptor affinities for these ligands are high, that these sites play a considerable role in cocaine-induced behavioral toxicity and locomotor activity. The contribution of σ_1 sites has recently been tested using σ receptor ligands with varying affinities for this σ receptor subtype (McCracken et al., 1998). It was determined that σ receptor ligands with low, micromolar affinities for σ_1 sites have a reduced attenuating capacity on convulsions induced by cocaine, while those with more favorable affinities have a greater attenuating effect (McCracken et al., 1998). In addition, antisense oligodeoxynucleotides for σ_1 receptors were capable of attenuating cocaine-induced convulsions and locomotor activity in preliminary studies (unpublished data). The σ_2 receptor subtype, on the other hand, has been shown to have a greater influence on psychomotor activities. Earlier studies indicate that the σ_2 receptor subtype participates in the mediation of locomotor activities such as circling and acute dystonic reactions (cf. Walker et al., 1994). Since BD1047 has a slightly lower affinity for σ_2 sites (47 ± 0.6 nM) than LR172 (2 ± 0.3 nM), this may explain the ability of a lower behaviorally inactive dose of LR172 to produce a comparable level of protection against the locomotor endpoint. Therefore, it appears that the σ_1 and σ_2 receptor subtypes both play a role in attenuating the actions of cocaine, but that the different subtypes have more or lesser of a role depending on the specific behaviors examined.

Together, the data suggest a role for σ receptor antagonists in treating cocaine overdose and addiction. BD1047 and LR172, like the parent compound BD1008, are σ receptor ligands with anti-cocaine properties. These σ receptor compounds, and the other analogs of BD1008 with similar characteristics, provide a basis for further development of new pharmacotherapies to treat the problems associated with the illicit use of cocaine.

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