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European Journal of Pharmacology 365 (1999) 35–38

ejp

Short communication

Novel σ receptor ligands attenuate the locomotor stimulatory effects of cocaine

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Received 7 September 1998; revised 23 November 1998; accepted 24 November 1998

Abstract

Cocaine interacts with σ receptors, suggesting that these sites are important for many of its behavioral effects. Therefore, two novel σ receptor ligands, BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine) and BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine), were evaluated for their ability to attenuate cocaine-induced locomotor activity. Receptor binding studies showed that BD1008 and BD1063 have nanomolar affinities for σ_1 and σ_2 sites, but a 250-fold or lower affinity for nine other receptors, making them among the most selective σ receptor ligands identified. In behavioral studies, pretreatment of mice with BD1008 or BD1063 produced a two-fold increase in the ED_{50} for the locomotor stimulatory effects of cocaine. These results suggest that σ receptors are involved in the behavioral effects of cocaine.

Keywords: Cocaine; Locomotor activity; Psychomotor effect

1. Introduction

One approach to treating cocaine addiction is to target the receptors that mediate its addicting properties. Cocaine is an indirect dopamine agonist and a potent inhibitor of the dopamine transporter (Kuhar et al., 1988). Therefore, dopamine receptors and the dopamine transporter system have historically been associated with some of the reinforcing and psychomotor effects of cocaine (Kuhar et al., 1988). However, cocaine is also known to interact with σ receptors at concentrations that are achievable in vivo (cf. Kuhar et al., 1988), indicating their possible role in reinforcing properties.

In earlier studies, putative σ receptor ligands/antagonists were reported to lessen the locomotor stimulatory effects of cocaine (Menkel et al., 1991; Witkin et al., 1993), but the compounds were non-selective, making it difficult to attribute their actions to σ receptors. With a more complete pharmacological understanding of these receptors, new, more selective ligands have since been developed. BD1008 is one such compound that has recently been reported to have anti-cocaine activities (Matsumoto et al., 1997). Its analog, BD1063, also possesses a high degree of selectivity and functional antagonist actions at σ receptors (Matsumoto et al., 1995). Therefore, BD1008 and BD1063 were tested for their ability to attenuate cocaine stimulated locomotor activity.

2. Materials and methods

2.1. Drugs

BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine) and BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine) were synthesized as previously described (De Costa et al., 1992, 1993). Cocaine hydrochloride was purchased from Sigma (St. Louis, MO, USA). The radioligands were obtained from Dupont/New England Nuclear (Boston, MA, USA) or

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00876-0

synthesized as described previously (De Costa et al., 1989). All of the chemicals used in the receptor binding assays were obtained from commercial sources, except for dextrallorphan, which was synthesized in the laboratory of Kenner C. Rice (NIDDK/NIH, Bethesda, MD, USA).

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 study took place.

2.3. Binding studies

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 receptors were labeled with [^{3}H](+)-pentazocine in the guinea pig brain; σ_2 sites were labeled in rat liver with [^{3}H]di-o-tolylguanidine (DTG) in the presence of a saturating concentration of dextrallorphan. Since many historic 'σ' ligands have non-specific interactions with receptors and binding sites, the relative selectivities of BD1008 and BD1063 were determined for dopamine, opioid, phencyclidine (PCP), α_1-adrenoceptors, α_2-adrenoceptors, β-adrenoceptors, 5-HT$_1$ and 5-HT$_2$ receptors. The methods used to obtain these affinities were previously published in detail (Bowen et al., 1993; Matsumoto et al., 1995). Briefly, dopamine D_2 receptors were labeled with [^{3}H](−)-sulpiride, opioid receptors were labeled with [^{3}H]etorphine, PCP sites were labeled with [^{3}H]1-[(2-thienyl)-cyclohexyl]piperidine (TCP), α_1-adrenoceptors were labeled with [^{3}H]prazosin, α_2-adrenoceptors were labeled with [^{3}H]clonidine, β-adrenoceptors were labeled with [^{3}H]dihydroalprenolol, 5-HT$_1$ receptors were labeled with [^{3}H]5-hydroxytryptamine, and 5-HT$_2$ receptors were labeled with [^{3}H]ketanserin.

2.4. Cocaine-induced locomotor activity

To measure locomotor activity, mice were acclimated for 30 min to the Plexiglas enclosures of an automated activity monitor (San Diego Instruments, San 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 arrays that border the Plexiglas enclosures.

Initially, 30 mg/kg doses of BD1008 and BD1063 were evaluated for their effects on locomotor activity because these doses produced significant protection against the behavioral toxic effects of cocaine in studies conducted earlier by this laboratory (Matsumoto et al., 1997). After a 30-min acclimation period, the animals were injected with 30 mg/kg (i.p.) of BD1008 ($n = 6$) or BD1063 ($n = 6$) and horizontal locomotor activity was measured for the following 30 min to ensure that these treatments produced effects no different from saline. In later experiments, an additional 1 mg/kg (i.p.) dose of BD1008 ($n = 6$) was also tested.

For the antagonism portion of the study, the mice were acclimated to the enclosures for 15 min. Then the animals were injected (i.p.) with saline or a behaviorally inactive dose of BD1008 (1 mg/kg or 30 mg/kg, $n = 64$) or BD1063 (30 mg/kg, $n = 30$). After the 15 min pretreatment period, varying doses of cocaine (0–20 mg/kg, i.p.) were administered and horizontal locomotor activity was quantified for the subsequent 30 min.

2.5. Statistics

The data from the binding assays was analyzed using GraphPad InPlot (San Diego, CA, USA). Apparent K_i values were calculated using the Cheng–Prusoff equation and K_d values previously determined (Matsumoto et al., 1990; Bowen et al., 1993; Hellewell et al., 1994). ED_{50} values for the cocaine dose curves were calculated using a locomotor activity score of 1769/30 min as the 50% value (50% of the mean locomotor activity after saline (0%) and the mean locomotor activity after the peak dose of cocaine (100%)).

Table 1
Affinities of BD1008 and BD1063 for σ receptors and other binding sites

	BD1008	BD1063[a]
σ Receptors		
σ_1	2 ± 1	9 ± 1
σ_2	8 ± 2	449 ± 11
Other receptors		
Dopamine D_2	1112 ± 74	> 10,000
Opioid	> 10,000	> 10,000
PCP	> 10,000	> 10,000
α_1	> 10,000	> 10,000
α_2	> 10,000	> 10,000
β	6783 ± 1861	> 10,000
5-HT$_1$	> 10,000	> 10,000
5-HT$_2$	> 10,000	2552 ± 2417

Affinities (in nM) were determined in competition binding assays, as described in Section 2. The affinities in the table represent K_i values for the σ receptor assays, which were calculated using K_d values that were determined in previous saturation assays. The affinities for the other receptors are represented as IC_{50}s. All of the values in the table represent the mean ± S.E.M. from a minimum of two experiments, each performed in duplicate. Values of > 10,000 nM signify that there was less than 30% displacement of the radioligand at this concentration.

[a] Data from the work of Matsumoto et al. (1995).

3. Results

3.1. Binding affinities

The K_i values for BD1008 and BD1063 at σ_1, σ_2, dopamine D_2, opioid, PCP, α_1, α_2, β, 5-HT_1 and 5-HT_2 receptors are indicated in Table 1. Both of the novel ligands have nanomolar affinities for σ receptors. In comparison, the ligands exhibited low to negligible affinities for the other sites.

3.2. Locomotor activity

Although when administered alone, BD1008 (1 mg/kg) and BD1063 (30 mg/kg) produced a level of locomotor activity that did not differ significantly from that of the saline vehicle (n.s.), when administered in combination with cocaine, both ligands attenuated cocaine-induced locomotor activity (Fig. 1). The ED_{50} value for the locomotor stimulatory effects of cocaine shifted from 6.50 mg/kg to 11.19 mg/kg in the presence of BD1008 (1 mg/kg), and 15.85 mg/kg in the presence of BD1063 (30 mg/kg).

When a higher, 30 mg/kg dose of BD1008 was tested, it produced effects that were no different from saline when administered alone (mean ± S.E.M. = 833 ± 119; n.s.). However, when combined with cocaine, this dose of BD1008 had marginal effects, shifting the ED_{50} for the dose curve for cocaine from 6.50 mg/kg to only 7.14 mg/kg. Further, when combined with the high 20 mg/kg dose of cocaine, BD1008 (30 mg/kg) enhanced cocaine stimulated locomotor activity (mean ± S.E.M. = 4235 ± 530).

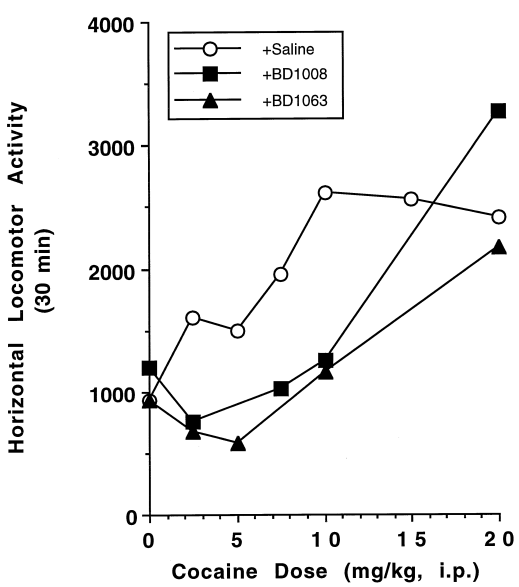

Fig. 1. Attenuation of the locomotor stimulatory effects of cocaine in the presence of behaviorally-inactive doses of BD1008 or BD1063. Mice were pretreated i.p. with saline, BD1008 (1 mg/kg), or BD1063 (30 mg/kg). After 15 min, they were injected i.p. with a dose of cocaine (0–20 mg/kg). Horizontal locomotor activity was quantified for the subsequent 30 min.

4. Discussion

The ability of BD1008 and BD1063 to protect against the locomotor stimulatory effects of cocaine are thought to be related to their actions at σ receptors. Both compounds have very high affinities for σ receptors and a 250-fold or weaker interaction with other receptors such as dopamine, opioid, PCP, α_1-adrenoceptors, α_2-adrenoceptors, β-adrenoceptors, 5-HT_1 and 5-HT_2 receptors. BD1008 is also known to be inactive at the dopamine transporter (unpublished data). The high selectivity of BD1008 and BD1063 for σ receptors thus suggests that they produce their actions through these proteins.

At this time, it is unclear whether the actions of BD1008 and BD1063 were mediated through σ_1 and/or σ_2 sites. However, it is worth noting that BD1008 and BD1063 have similar affinities for σ_1 receptors and varying affinities for σ_2 receptors. The σ_2 subtype is thought to mediate locomotor activities such as circling and acute dystonic reactions (cf. Walker et al., 1994). Since BD1008 has a much higher affinity for σ_2 sites than BD1063, this could explain the ability of a lower behaviorally inactive dose of BD1008 to produce a comparable level of protection.

Further, the affinity of BD1008 for dopamine and β-adrenoceptors, although over a 100-fold lower than its affinity for σ receptors, is still within a range where it can produce physiologically relevant actions. Therefore, the observation that the higher 30 mg/kg dose of BD1008 had a weaker attenuating effect against the locomotor stimulatory actions of cocaine when compared to the lower 1 mg/kg dose, may be due to the activation of monoaminergic systems (Harris et al., 1996). This complication is not seen with BD1063, a compound that lacks significant interactions with monoaminergic receptors.

Together with results previously published by others, the data suggest that σ receptors can mediate the psychomotor stimulatory effects of cocaine. Additional studies are warranted to fully characterize the nature of these interactions.

Acknowledgements

We thank Dr. Brian de Costa (NIDDK, NIH, Bethesda, MD) for synthesizing BD1008 and BD1063. The National Institutes of Mental Health (MH50564) supported this work.

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