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The Acute Administration of the Selective Dopamine D₃ Receptor Antagonist SB-277011A Reverses Conditioned Place Aversion Produced by Naloxone Precipitated Withdrawal From Acute Morphine Administration in Rats

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Abstract

We examined the effect of SB-277011A, a selective D₃ receptor antagonist, on the conditioned place aversion (CPA) response associated with naloxone-induced withdrawal from acute morphine administration in male Sprague-Dawley rats. Morphine (5.6 mg/kg i.p.) was given, followed 4 hrs later by naloxone (0.3 mg/kg i.p.) and prior to placing the animals in one specific chamber of the test apparatus. All animals were subjected to 2 of these trials. A significant CPA occurred in animals that received an i.p. injection of vehicle 30 minutes prior to the measurement of chamber preference. The pretreatment of animals (30 minutes prior to testing) with 3 mg/kg i.p. of SB-277011A did not significantly alter the CPA compared to animals treated with vehicle (1 ml/kg i.p. of deionized distilled water). In contrast, the acute pretreatment of animals with 6, 12 or 24 mg/kg i.p. of SB-277011A significantly decreased the CPA compared to vehicle-treated animals. In fact, the 12 and 24 mg/kg doses of SB-277011A significantly increased the time spent in the chamber where animals were paired with morphine and naloxone. These results suggest that the selective antagonism of D₃ receptors attenuates the CPA produced by a model of naloxone-induced withdrawal from acute morphine dependence.

Keywords

Dopamine; D₃ receptors; SB-277011A; conditioned place aversion; naloxone-induced withdrawal

Behavioral studies in rodents have shown that the acute administration of the highly selective dopamine (DA) D₃ receptor antagonist SB-277011A significantly attenuates the appetitive and reinforcing effects produced by various addictive drugs (for comprehensive

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reviews, see Heidbreder et al., 2005; Heidbreder and Newman, 2010), including nicotine (Andreoli et al., 2003; Pak et al., 2006), cocaine (Vorel et al., 2002; Xi et al., 2005), heroin (Ashby et al., 2003), methamphetamine (Spiller et al., 2008; Higley et al., 2011), and ethanol (Heidbreder et al., 2007; Vengeliene et al., 2006). In addition, SB-277011A significantly decreases cue- (Gilbert et al., 2005), stress- (Xi et al., 2004), and drug-induced (Vorel et al., 2002) reinstatement of cocaine-seeking behavior in rats. In contrast, the systemic administration of SB-277011A does not have rewarding or reinforcing properties in rodents (Vorel et al., 2002; Xi et al., 2005). Furthermore, it does not significantly impair memory or psychomotor function at doses that significantly attenuate the rewarding or reinforcing actions of addictive drugs (Reavill et al., 2000). These aforementioned properties of SB-277011A, provided they can be extrapolated to humans, tentatively suggest that selective DA D₃ receptor antagonists may be used in the treatment of drug addiction. However, one aspect of treating addiction that requires consideration is whether the treatment being used may produce significant dysphoric and/or aversive effects, thereby potentially decreasing treatment compliance. In addition, treatments that exacerbate withdrawal symptoms after discontinuation of drug self-administration would not be desirable. Currently, it is unknown as to whether the systemic administration of DA D₃ receptor antagonists would produce significant changes in animals that are conditioned to aversive effects. Therefore, in this study, we examined the effect of SB-277011A on the conditioned place aversion (CPA) caused by naloxone in adult male rats conditioned to the incentive motivational effects of morphine.

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), 150–175 g on arrival, were used for all experiments. The animals were allowed to acclimate to the vivarium environment for 3 days before handling. The animals were handled for 5 min per day for 4 days before the CPA experiments. A three-chambered place preference, as previously described (Vorel et al., 2002) was used. The experimental design and procedure was similar to that of Azar et al. (2003), except that a preconditioning phase was not used. In the conditioning phase, animals ($n = 10$ per experimental group) received injections (1 ml/kg) of vehicle (deionized, distilled water) or naloxone (0.3 mg/kg s.c. dissolved in vehicle) 4 h after receiving either vehicle or morphine (5.6 mg/kg i.p. dissolved in deionized, distilled water). Subsequently, the animals were confined to the predetermined chambers to be paired with that drug condition for 20 min. Animals were subjected to two cycles of conditioning and were given vehicle/vehicle on Days 1 and 3 and morphine/naloxone on Days 2 and 4, and the test day was conducted 24 h later. On the test day (Day 5), animals received either vehicle or SB-277011A (3, 6, 12, or 24 mg/kg i.p. dissolved in 2% methylcellulose) and 30 min later, were allowed access to the entire apparatus for 15 min. The time spent in each chamber was recorded using an automated device. The data were expressed as the raw time spent in each chamber. The data were analyzed using a one-way ANOVA and post hoc analyses were conducted using the Student-Newman-Keuls test. Morphine, naloxone, and methyl-cellulose were purchased from Sigma Chemicals (St. Louis, MO). SB277011A was obtained as a gift from GlaxoSmithKline (Verona, Italy).

Statistical analyses indicated a significant effect of treatment on the CPA response ($F_{(4,45)} = 39.15$, $P < 0.0001$, Table I). Subsequent post-hoc tests indicated that CPA was significantly reduced by a single i.p. injection of SB-277011A 6 mg ($q = 44.2$), 12 mg ($q = 82.3$), or 24

mg ($q = 96.2$) compared with animals treated with vehicle (Table I). Animals that were paired with vehicle + naloxone and received vehicle on the test day spent more time on the paired side compared with animals paired with morphine and naloxone and injected with vehicle on the test day (Student's unpaired t test, $t = 6.28$, $P < 0.0001$). This indicates that the aversion produced by naloxone in the morphine-paired animals is due to its antagonism of opioid receptors, thereby precipitating withdrawal and producing a CPA.

As previously reported by Azar et al. (2003), the administration of naloxone 4 h before morphine for one cycle produced a significant CPA in adult male rats. Overall, the results of this study indicate that in male Sprague-Dawley rats a single i.p. injection of 6, 12, or 24 mg/kg of SB-277011A significantly decreases the CPA produced by s.c. naloxone in morphine-paired animals. It is possible that SB-277011A's effect is related to it eliciting a rewarding effect, thus causing the animals to spend more time away from the chamber associated with naloxone-induced aversion. However, this is unlikely as (1) the acute i.p. administration of SB-277011A does not significantly alter brain stimulation reward threshold in rats (Vorel et al., 2002); (2) SB-277011A by itself does not produce conditioned place preference (Vorel et al., 2002; Pak et al., 2006), and (3) animals self-administer SB-277011A at a rate similar to that of vehicle (i.e., SB-277011A is not appetitive) (Xi et al., 2005). It is unlikely that SB-277011A's effect results from an impairment of memory as it enhances social recognition in rats (Loiseau and Millan, 2009; Millan et al., 2007), prevents scopolamine-induced learning deficits without altering the normal learning process in non-impaired rats (Laszy et al., 2005), and increases extracellular levels of acetylcholine in frontocortical regions (Lacroix et al., 2003, 2006; Millan et al., 2007). In addition, at doses used in this study, SB-277011A does not produce D_2 antagonistic actions (Reavill et al., 2000; Heidbreder et al., 2005), indicating that our results are not related to its interaction with D_2 receptors. Finally, the reversal of naloxone-induced CPA by SB-277011A could be due to an anxiolytic effect. However, there have been no published reports indicating that highly selective D_3 receptor antagonists, such as SB-277011A, exert anxiolytic actions. There is, however, evidence showing that SB-277011A attenuates the expression of fear conditioning as assessed by conditioned suppression in the rat, suggesting that selective antagonism at DA D_3 receptors blocks the ability of aversive conditioned stimuli to affect behavior (Swain et al., 2008).

In conclusion, the precipitation of withdrawal by naloxone in rats paired with morphine was significantly attenuated by the acute administration of 6, 12, or 24 mg/kg of SB-277011A. The mechanism responsible for this effect remains to be elucidated. These results, provided they can be extrapolated to humans, suggest that highly selective D_3 antagonists could attenuate some of the aversive effects that result from acute opioid withdrawal.

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TABLE I

The effect of a single i.p. administration of vehicle and SB-277011A on the conditioned place aversion induced by a two-cycle pairing of morphine + naloxone in adult male Sprague-Dawley rats

Treatment pairings	Drug given p.o. on test day	Time spent in chambers (min)	
		Paired	Unpaired
Morphine/Naloxone	Vehicle ^a	5.5 ± 0.3 ^b	9.5 ± 0.3
Vehicle/Naloxone	Vehicle ^a	7.1 ± 0.2 ^c	7.9 ± 0.2
Morphine/Naloxone	SB, 3 mg/kg	6.3 ± 0.4	8.7 ± 0.4
Morphine/Naloxone	SB, 6 mg/kg	8.8 ± 0.3 ^d	6.2 ± 0.3
Morphine/Naloxone	SB, 12 mg/kg	10.0 ± 0.3 ^e	5.0 ± 0.3
Morphine/Naloxone	SB, 24 mg/kg	10.3 ± 0.4 ^e	4.7 ± 0.4

Abbreviations: SB: SB-277011A.

^aThe vehicle was 1 ml/kg of distilled water for the pairings. The vehicle used on the test day was 1 ml/kg i.p. of 2% methylcellulose.

^bEach value represents the mean number of minutes spent in each chamber ± S.E.M. A total of 10 rats were examined for each treatment pairing. All animals received one pairing with 5.6 mg/kg i.p. of morphine followed 4 h later by 0.3 mg/kg s.c. of naloxone followed 24 h later by 1 pairing with vehicle 1 ml/kg i.p. and s.c. of vehicle (deionized, distilled water), and this was repeated once over 4 days. All animals were confined to the appropriate chamber for 20 min during each pairing. On the test day, animals received either 1 ml/kg i.p. vehicle (2% methylcellulose) or SB-277011A (dissolved in 2% methylcellulose) 30 min before being placed into the CPP apparatus. The times spent in paired chamber represents the time spent in the compartment originally paired with morphine + naloxone or vehicle + naloxone.

^cSignificantly greater than Morphine/Naloxone pairings and Vehicle on the test day, $P < 0.0001$, Student's unpaired t test.

^dSignificantly greater than Morphine/Naloxone pairings and vehicle on the test day and Morphine/Naloxone pairings and 3 mg/kg of SB-277011A on the test day, $P < 0.01$, ANOVA and Student-Newman-Keuls test.

^eSignificantly greater than Morphine/Naloxone pairings and Vehicle on the test day ($P < 0.01$), Morphine/Naloxone pairings and 3 mg/kg of SB-277011A on the test day ($P < 0.01$) and Morphine/Naloxone pairings and 6 mg/kg of SB-277011A on the test day ($P < 0.05$), ANOVA and Student-Newman-Keuls test.