The Orexin-1 Antagonist SB-334867 Blocks Antipsychotic Treatment–Emergent Catalepsy: Implications for the Treatment of Extrapyramidal Symptoms

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We have previously shown that the orexin-1 antagonist SB-334867 blocks the electrophysiological effects of haloperidol and olanzapine on the activity of A9 and A10 dopamine neurons. To evaluate if orexin-1 antagonists might block other effects of antipsychotic drugs in animals, we examined the effects of SB-334867 on behavioral, neurochemical, and neuroendocrine effects of antipsychotic drugs. Pretreatment with SB-334867 (0.01–10 mg/kg, intraperitoneal [IP]) significantly decreased the catalepsy produced by the administration of haloperidol (1 mg/kg, subcutaneous [SC]), risperidone (2 mg/kg, SC), and olanzapine (10 mg/kg, SC). Administration of SB-33467 also reversed catalepsy after it had been established in animals pretreated 2 hours earlier with haloperidol. However, pretreatment with SB-334867 (1–10 mg/kg, IP) did not block the decreases in exploratory locomotor activity produced by administration of haloperidol (0.1 mg/kg, SC) or risperidone (0.3 mg/kg, SC). In addition, pretreatment with SB-334867 (1–10 mg/kg, IP) neither blocked the increased levels of dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens or striatum nor the elevation in serum prolactin produced by administration of haloperidol (0.1 mg/kg, SC) and risperidone (1 mg/kg, SC). Administration of SB-334867 alone neither changed locomotor activity and DOPAC or prolactin levels nor produced catalepsy. These results show that orexin-1 antagonists block the catoleptogenic effects of antipsychotics but do not block other locomotor, neurochemical, or neuroendocrine effects of antipsychotics. Because catalepsy is thought to be a good predictor of extrapyramidal symptoms in humans, treatment with orexin-1 antagonists might decrease the occurrence or severity of antipsychotic treatment–emergent extrapyramidal symptoms in humans.

Key words: antipsychotics/orexin/catalepsy/olanzapine/haloperidol/risperidone

Orexin-A and orexin-B (also known as hypocretin 1 and hypocretin 2, respectively) are peptide neurotransmitters derived from the precursor prepro-orexin.¹ Receptors for orexin-A and orexin-B are G-protein coupled and are divided into 2 types: orexin-1 and orexin-2. The cell bodies of orexin-containing neurons are located almost exclusively in the lateral hypothalamus and send extensive projections throughout the brain. The orexin system has been hypothesized to play a role in a number of brain functions including modulation of the neuroendocrine system, control of feeding and energy metabolism, and regulation of the sleep-wake cycle.¹–⁵ In addition, the orexin system has been shown to interact with the brain’s dopamine system. For example, there is a dense projection of orexin neurons from the lateral hypothalamus to A10 dopamine neurons,⁶ and some antipsychotics have been shown to increase the expression of c-fos in orexin-containing cells in the hypothalamus.⁷ In addition, direct application of orexins can activate at least a subpopulation of A10 dopamine neurons,⁸ and orexin-A induces a potentiation of N-methyl-D-aspartate–mediated neurotransmission in A10 dopamine neuron synapses.⁹

Recently, the orexin-1 antagonist SB-334867 has been shown to block the electrophysiological effects of haloperidol and olanzapine on the activity of A9 and A10 dopamine neurons.¹⁰ Because the effects of antipsychotic drugs on dopamine neuronal activity have been hypothesized to play a role in their clinical effects, these data indicate that orexin-1 antagonists might affect some clinical effects of antipsychotic drugs. To evaluate if orexin-1 antagonists might block other effects of antipsychotic drugs in animals, we examined the effects of SB-334867 on some behavioral (catalepsy and exploratory locomotor activity), neurochemical (elevation of dopamine metabolites), and neuroendocrine (elevation of serum prolactin) effects of antipsychotic drugs.

Methods

Animals

Male, Sprague-Dawley rats (200–300 g; Harlan Industries, Indianapolis, IN) were group housed with food
and water available ad lib. A 12:12 light:dark cycle was maintained in the colony room. All procedures were carried out in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and were approved by the Eli Lilly Animal Care and Use Committee.

Drug Solutions

Olanzapine (Eli Lilly & Co, Indianapolis, IN), risperidone (MP Biomedical Inc, Solon, OH), and haloperidol (Sigma, St. Louis, MO) were dissolved in a combination of 5% lactic acid, sodium hydroxide, and distilled water (final pH = 6–7). SB-334867 (Eli Lilly & Co, Indianapolis, IN) was dissolved in 50% (2-hydroxypropyl)-β-cyclo-dextrin (Sigma, St. Louis, MO). In all tests, doses of antipsychotics were chosen to produce an intermediate level of activity, so that decreases or increases in the activity could be detected following pretreatment with SB-334867.

Behavior

Catalepsy was measured using the bar test. Rats’ front limbs were placed over a 2-cm high horizontal bar. The intensity of the catalepsy was measured by the time the rats remained in this position on 3 consecutive trials (maximum 120 seconds). The longest time of the 3 trials was used for the catalepsy score. Doses of antipsychotics were chosen to produce an intermediate level of activity, so that decreases or increases in the activity could be detected following pretreatment with SB-334867. Animals were pretreated with vehicle (1 mL/kg, intraperitoneal [IP]) or SB-334867 (0.01–10 mg/kg, IP) 15 minutes before administration of haloperidol (1 mg/kg, subcutaneous [SC]), risperidone (2 mg/kg, SC), olanzapine (10 mg/kg, SC), or vehicle (1 mL/kg). Catalepsy was measured at 1 and 2 hours following the second injection. A separate group of animals not only received haloperidol or vehicle and had catalepsy measured at 1- and 2-hour intervals for 4 hours but also received an injection of haloperidol (0.1 mg/kg, SC), risperidone (0.3 mg/kg, SC), or vehicle (1 mL/kg, SC). Rats were sacrificed 90 minutes after the second injection, and trunk blood was collected in chilled tubes containing 40 μL 0.5 M EDTA and centrifuged at 3000 rpm at 4 °C for 10 minutes. Plasma was isolated and stored at −40 °C prior to assay for prolactin using rat SPIBio prolactin enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Striatum and nucleus accumbens were dissected and stored frozen at −40 °C prior to measures of monoamines and metabolites by high-pressure liquid chromatography-electrochemical detection. Statistical differences were identified using analysis of variance followed by Dunnett’s Test post hoc.

Results

Catalepsy

Pretreatment with SB-334867 (1–10 mg/kg, IP), but not vehicle, significantly decreased the catalepsy produced by the administration of haloperidol (figure 1, n = 6 per group). Pretreatment with SB-334867 (0.03–0.1 mg/kg, IP), but not vehicle, also significantly decreased the catalepsy produced by the administration of risperidone (figure 1, n = 5–6 per group); doses up to 3 mg/kg SB-334867 did not produce any greater blockade (data not shown). In addition, pretreatment with 0.03 and 0.1 mg/kg SB-334867 (IP), but not vehicle or 0.01 mg/kg SB-334867, significantly decreased the catalepsy produced by the administration of olanzapine (figure 3, n = 5–6 per group). Pretreatment with SB-334867 (0.3, 3, or 10 mg/kg, IP) alone had no effect on catalepsy scores (figure 1). Administration of SB-334647 (10 mg/kg, IP), but not vehicle, also reversed catalepsy after it had been established in animals pretreated 2 hours earlier with haloperidol (1 mg/kg, SC) (figure 2, n = 6 per group).
Pretreatment with SB-334867 (0.3–10 mg/kg, IP) did not block the decrease in exploratory locomotor activity produced by administration of haloperidol (0.1 mg/kg, SC; figure 3, \( n = 5–12 \) per group) or risperidone (0.3 mg/kg, SC; figure 3, \( n = 6–12 \) per group). Administration of 3 or 10 mg/kg SB-334867 alone did not affect exploratory locomotor activity.

**DOPAC**

Pretreatment with SB-334867 (1–10 mg/kg, IP) or vehicle did not block the increased levels of DOPAC in the striatum (figure 4, \( n = 5 \) per group) or nucleus accumbens (figure 4, \( n = 5 \) per group) produced by administration of haloperidol (0.1 mg/kg, SC). Pretreatment with 0.3 mg/kg SB-334867 (IP), 3 mg/kg SB-334867 (IP), or vehicle did not block the increased levels of DOPAC in the striatum (figure 5, \( n = 5 \) per group) produced by administration of risperidone (1 mg/kg, SC). Pretreatment with 1 mg/kg SB-334867 (IP) produced DOPAC levels in the striatum that were slightly, but significantly, lower than pretreatment with vehicle; however, the DOPAC levels were still significantly above vehicle/vehicle control (figure 5). Pretreatment with 0.3 mg/kg SB-334867 (IP) lead to levels of DOPAC in the nucleus accumbens that were slightly, but significantly, below vehicle/vehicle and vehicle/risperidone animals (figure 5, \( n = 5 \) per group). Pretreatment with 1 or 3 mg/kg SB-334867 (IP) lead to levels of DOPAC in the nucleus accumbens that were slightly, but significantly, above vehicle/risperidone control (figure 5). Administration of 3 or 10 mg/kg SB-334867 alone did not alter DOPAC levels in the striatum or nucleus accumbens.

**Prolactin**

Pretreatment with SB-334867 (0.3–10 mg/kg, IP) did not block the increase in serum prolactin levels produced by administration of haloperidol (0.1 mg/kg, SC; figure 6, \( n = 4–5 \) per group) or risperidone (0.3 mg/kg, SC; figure 6, \( n = 5 \) per group). Administration of 3 or 10 mg/kg SB-334867 alone did not affect serum prolactin levels.
In agreement with previous studies, administration of haloperidol, risperidone, and olanzapine produced catalepsy in rats. Pretreatment with the orexin-1 antagonist SB-334867 blocked the catoleptogenic effects of haloperidol, risperidone, and olanzapine (figure 1). In addition, administration of SB-334867 significantly decreased the severity of catalepsy in haloperidol-treated animals already displaying strong catalepsy (figure 2). Because catalepsy is thought to be a good predictor of extrapyramidal symptoms in humans, treatment with orexin-1 antagonists might decrease the occurrence or severity of antipsychotic treatment–emergent extrapyramidal symptoms in humans. Multiple ways of measuring catalepsy have been reported (eg, cross-legged position, electromyographic activity). It would be interesting to see if SB-334867 also blocked the effects of antipsychotics using other measures of catalepsy.

We also examined the effects of SB-334867 on another motor output that is known to be affected by antipsychotic drugs: exploratory locomotor activity. As has been shown previously, administration of haloperidol and risperidone produced a decrease in locomotor activity. Doses of SB-334867 that were able to completely block the cataleptogenic effects of haloperidol and risperidone did not affect the decreased locomotor activity produced by these compounds (figure 3). These data indicate that the blockade of catalepsy by SB-334867 is not due to a general decrease of all antipsychotic-induced changes in motor-related behaviors. This hypothesis could be further explored by examining the effect of orexin-1 antagonists on other antipsychotic-induced electrophysiological effects of antipsychotic drugs on nigrostriatal (A9) dopamine neurons. Output from the striatum is known to play an important role in the effects of antipsychotic drugs on A9 dopamine cells because acute transection of the striatonigral afferents will reverse antipsychotic-induced depolarization inactivation and restore A9 activity. Thus, changes in neuronal activity in the striatum may be a final common pathway for the effects of SB-334867 on both antipsychotic-induced catalepsy and antipsychotic-induced changes in A9 neuronal activity. Additional detailed studies of the effects of orexin-1 antagonists on striatal neuronal activity would help address this hypothesis.

Discussion

In agreement with previous studies, administration of haloperidol, risperidone, and olanzapine produced catalepsy in rats. Pretreatment with the orexin-1 antagonist SB-334867 blocked the catoleptogenic effects of haloperidol, risperidone, and olanzapine (figure 1). In addition, administration of SB-334867 significantly decreased the severity of catalepsy in haloperidol-treated animals already displaying strong catalepsy (figure 2). Because catalepsy is thought to be a good predictor of extrapyramidal symptoms in humans, treatment with orexin-1 antagonists might decrease the occurrence or severity of antipsychotic treatment–emergent extrapyramidal symptoms in humans. Multiple ways of measuring catalepsy have been reported (eg, cross-legged position, electromyographic activity). It would be interesting to see if SB-334867 also blocked the effects of antipsychotics using other measures of catalepsy.

The blockade of nigrostriatal dopamine transmission has been hypothesized to play an important role in antipsychotic-induced catalepsy. Interestingly, SB-334867 blocks both antipsychotic-induced catalepsy and the
changes in motor behavior (eg, disruption of rotorod performance).

We also examined the effects of SB-334867 on some neurochemical and neuroendocrine changes produced by the administration of antipsychotics. Dopamine D2 receptor antagonists increase the release of dopamine into the synapse due to blockade of the D2 autoreceptor.16 In this situation, increases in the levels of the dopamine metabolites DOPAC and homovanillic acid reflect increased neuronal dopaminergic activity in vivo. For example, olanzapine and other dopamine D2 receptor antagonists increase concentrations of DOPAC in the striatum and nucleus accumbens.16,17 Pretreatment with SB-334867 had no effect on the increased DOPAC levels in the striatum or nucleus accumbens produced by haloperidol (figure 4). Pretreatment with SB-334867 had modest effects on the increased DOPAC levels in the striatum and nucleus accumbens produced by risperidone. However, these effects were weak and inconsistent and never prevented a significant increase in DOPAC levels by risperidone (figure 5). Antipsychotics also produce an elevation in circulating prolactin levels in rats and humans.18,19 Prolactin elevation results from D2 receptor blockade on anterior pituitary lactotrophs, where dopamine exerts a tonic inhibitory effect on prolactin secretion.20 Serum prolactin levels were measured in the same animals in which DOPAC levels were examined. Pretreatment with SB-334867 had no effect on increased serum prolactin levels produced by haloperidol or risperidone administration (figure 6). Thus, SB-334867 did not block several measures of the D2 antagonist activity of haloperidol and risperidone.

The mechanism of the blockade of antipsychotic-induced catalepsy by SB-334867 cannot be determined from this study. Because SB-334867 does not block several other measures of D2 antagonist activity (ie, DOPAC accumulation, prolactin elevation), it is unlikely that SB-334867 alters the binding of haloperidol, risperidone, or olanzapine to D2 receptors. Thus, it appears likely that antagonism of orexin-1 receptors interrupts the effects of D2 receptor blockade “downstream” from (ie, distal to) the D2 receptor. However, of the effects studied so far, this blockade appears to be selective for the cataleptogenic effects of antipsychotics. Moderate levels of orexin-1 receptors have been shown to exist in the basal ganglia.21 Given the potential role of the basal ganglia in catalepsy, one possibility is that blockade of orexin-1 receptors in the basal ganglia may play a role in the blockade of antipsychotic-induced catalepsy.

Fig. 5. Effect of pretreatment with SB-334867 (0.3–3 mg/kg, IP) or vehicle (Veh) 15 minutes before administration of risperidone (Ris; 1 mg/kg, subcutaneous) or Veh on DOPAC levels in the striatum (top) or nucleus accumbens (bottom) measured 90 minutes post risperidone administration. n = 5 per group. ***P < .001 vs Veh/Veh; ###P < .001 vs Veh/1 Ris.

Fig. 6. Top: Effect of pretreatment with SB-334867 (1–10 mg/kg, IP) 15 minutes before administration of haloperidol (Hal; 0.1 mg/kg, subcutaneous [SC]) on serum prolactin levels measured 90 minutes post haloperidol administration. n = 4–5 per group. *P < .05, ***P < .001 vs vehicle (Veh)/Veh; #P < .05 vs Veh/0.1 Hal. Bottom: Effect of pretreatment with SB-334867 (0.3–3 mg/kg, IP) 15 minutes before administration of risperidone (Ris; 1 mg/kg, SC) on serum prolactin levels measured 90 min post risperidone administration. n = 5 per group. **P < .01, ***P < .001 vs vehicle (Veh)/Veh; ##P < .01, ###P < .001 vs Veh/1 Ris.
While haloperidol is relatively selective for dopamine D2, D3, and D4 receptors, many antipsychotic drugs (including risperidone and olanzapine) have a more complex pharmacology. These compounds produce a number of well-documented effects in animals, some of which are thought to be predictive of their clinical efficacy. Additional work will be needed to determine if orexin-1 receptor antagonists can alter the effects of antipsychotic drugs in animal models thought to be predictive of their efficacy (e.g., conditioned avoidance responding). In this regard, specific patterns of changes in c-fos expression have been hypothesized as one marker of antipsychotic efficacy.22–24 A preliminary report indicates that orexin-1 receptor antagonists can block the effects of clozapine on Fos expression in the prefrontal cortex.25 Whether orexin-1 receptor antagonists can block the effects of antipsychotics on Fos expression in other brain areas (e.g., nucleus accumbens) will need to be determined. In addition, tests with other selective orexin-1 antagonists are needed to confirm the pharmacological specificity of the effects reported here.

In conclusion, the orexin-1 antagonist SB-334867 blocked catalepsy produced by haloperidol, risperidone, and olanzapine. However, SB-334867 neither blocked the decreases in exploratory locomotor activity and increased levels of DOPAC in the nucleus accumbens or striatum nor the elevation in serum prolactin produced by administration of haloperidol or risperidone. Administration of SB-334867 alone neither changed locomotor activity and DOPAC or prolactin levels nor produced catalepsy. These results show that an orexin-1 antagonist blocks the catalepticogenic effects of antipsychotics but does not block other locomotor, neurochemical, or neuroendocrine effects of antipsychotics. Because catalepsy is thought to be a good predictor of extrapyramidal symptoms in humans, treatment with orexin-1 antagonists might decrease the occurrence or severity of antipsychotic treatment–emergent extrapyramidal symptoms in humans.

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