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Atypical antipsychotic properties of blonanserin, a novel dopamine D_2 and 5-HT_{2A} antagonist

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ABSTRACT

Blonanserin is a novel antipsychotic agent that preferentially interacts with dopamine D_2 and 5-HT_{2A} receptors. To assess the atypical properties of blonanserin, we evaluated its propensity to induce extrapyramidal side effects (EPS) and to enhance forebrain Fos expression in mice. The actions of AD-6048, a primary metabolite of blonanserin, in modulating haloperidol-induced EPS were also examined. Blonanserin (0.3–10 mg/kg, p.o.) did not significantly alter the pole-descending behavior of mice in the pole test or increase the catalepsy time, while haloperidol (0.3–3 mg/kg, p.o.) caused pronounced bradykinesia and catalepsy. Blonanserin and haloperidol at the above doses significantly enhanced Fos expression in the shell (AcS) region of the nucleus accumbens and dorsolateral striatum (dlST). The extent of blonanserin-induced Fos expression in the AcS was comparable to that induced by haloperidol. However, the striatal Fos expression by blonanserin was less prominent as compared to haloperidol. Furthermore, combined treatment of AD-6048 (0.1–3 mg/kg, s.c.) with haloperidol (0.5 mg/kg, i.p.) significantly attenuated haloperidol-induced bradykinesia and catalepsy. The present results show that blonanserin behaves as an atypical antipsychotic both in inducing EPS and enhancing forebrain Fos expression. In addition, AD-6048 seems to contribute at least partly to the atypical properties of blonanserin.

1. Introduction

Schizophrenia is a psychiatric disorder with diverse symptoms including positive symptoms (e.g., hallucinations, delusion and excitation), negative symptoms (e.g., apathy, social and emotional withdrawal) and cognitive impairments (Lewine et al., 1983; Crow, 1985). A variety of agents (e.g., phenothiazines, butyrophenones and benzamides) which commonly possess dopamine D_2 blocking activity have long been used to treat patients with schizophrenia. However, these typical antipsychotics frequently induce extrapyramidal side effects (EPS) such as akathisia, tremor, hypokinesia (e.g., bradykinesia and akinesia) and are poorly effective against negative symptoms or cognitive impairments (Meltzer and Nash, 1991; Ohno et al., 1997; Meltzer et al., 2003).

Previous studies have revealed the therapeutic role of 5-HT_{2A} receptors in schizophrenia, indicating that 5-HT_{2A} receptor antagonism can reduce antipsychotics-induced EPS and ameliorates the negative symptoms (Bleich et al., 1988; Meltzer and Nash, 1991; Ohno et al., 1997; Meltzer et al., 2003). Based on this "serotonin hypothesis in schizophrenia", a line of atypical antipsychotics (e.g., risperidone, perospirone, olanzapine and quetiapine) that combine 5-HT_{2A} and D_2 antagonistic actions has been developed in the past two decades. These agents commonly show higher binding affinity to 5-HT_{2A} receptors than to D_2 receptors (Meltzer and Nash, 1991; Meltzer et al., 2003).

Blonanserin is a novel antipsychotic drug that preferentially interacts with D_2 and 5-HT_{2A} receptors (Une and Kurumiya, 2007). It showed efficacy in various animal models (e.g., methamphetamine- or phencyclidine (PCP)-induced hyperactivity) predictive for the positive symptoms in schizophrenia with a potency similar to that of haloperidol (Oka et al., 1993; Noda et al., 1993; Nagai et al., 2003). In addition, blonanserin was reported to alleviate PCP-induced immobility in the forced swim test, implying its efficacy for the negative symptoms (Nagai et al., 2003). Nonetheless, information on the EPS liability of blonanserin and its mechanism is limited and it remains inconclusive whether blonanserin behaves as an atypical agent due to species differences and a bell shaped dose–response in its EPS induction (Oka et al., 1993). Furthermore, blonanserin is distinct from the current 5-HT_{2A} and D_2 antagonists in that it exhibits higher affinity to D_2 receptors than to 5-HT_{2A} receptors (Une and Kurumiya, 2007).

Therefore, in order to assess the EPS liability of blonanserin, we compared its propensities to induce EPS (i.e., bradykinesia and catalepsy) and to enhance forebrain Fos expression with those of haloperidol in mice. In addition, we evaluated the anti-EPS actions of

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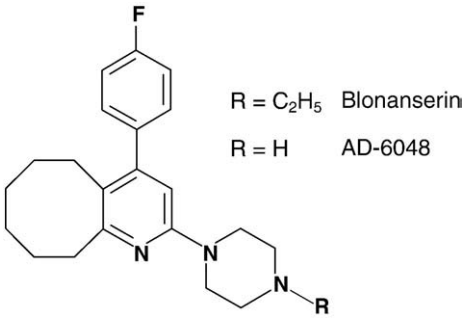

Fig. 1. Chemical structures of blonanserin and its metabolite AD-6048.

AD-6048, a primary metabolite of blonanserin, to clarify its potential role in modulating EPS.

2. Materials and methods

2.1. Animals

Male ddY mice (Japan SLC, Shizuoka, Japan) weighing 25–35 g were used. The animals were kept in air-conditioned rooms under a 12-h light/dark cycle and allowed *ad libitum* access to food and water. The housing conditions of the mice and the animal care methods complied with the NIH guide for the care and use of laboratory animals. The experimental protocols of this study were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

2.2. Pole test

Pole test was carried out as described previously (Ohno et al., 1994, 2008a). Briefly, mice were placed head-upward at the top of a wooden pole (8 mm in diameter and 45 cm in height), and then the time for the animal to rotate downward completely (T_{turn}) and descend to the floor (T_{total}) was measured with a maximum limit of 90 s. Bradykinesia was evaluated as the prolongation of T_{turn} and T_{total} values. Blonanserin (0.3–10 mg/kg), haloperidol (0.3–3 mg/kg) or a vehicle (Control) was orally administered to the mice 30 min before the pole test. In the experiments examining the antibradykinetic effects of AD-6048, animals were first treated with AD-6048 (0.1–3 mg/kg, s.c.), and 15 min later, treated with 0.5 mg/kg (i.p.) of haloperidol. Pole test was carried out 30 min after the haloperidol injection.

2.3. Catalepsy test

Catalepsy test was carried out as described previously (Ohno et al., 2008a, 2009). Briefly, the forepaws of the animals were placed on a horizontal bar positioned 5 cm above the bench surface, and the time spent for the animals to show a cataleptic posture, which was defined as an immobile posture while keeping both forelimbs on the bar, was measured with a maximum limit of 180 s. Blonanserin (0.3–10 mg/kg), haloperidol (0.3–3 mg/kg) or a vehicle (Control) was orally administered to the mice 30 min before the catalepsy test. In the experiments examining the anticataleptic effects of AD-6048, animals were pretreated with AD-6048 (0.1–3 mg/kg, s.c.) 15 min before the haloperidol (0.5 mg/kg, i.p.) injection.

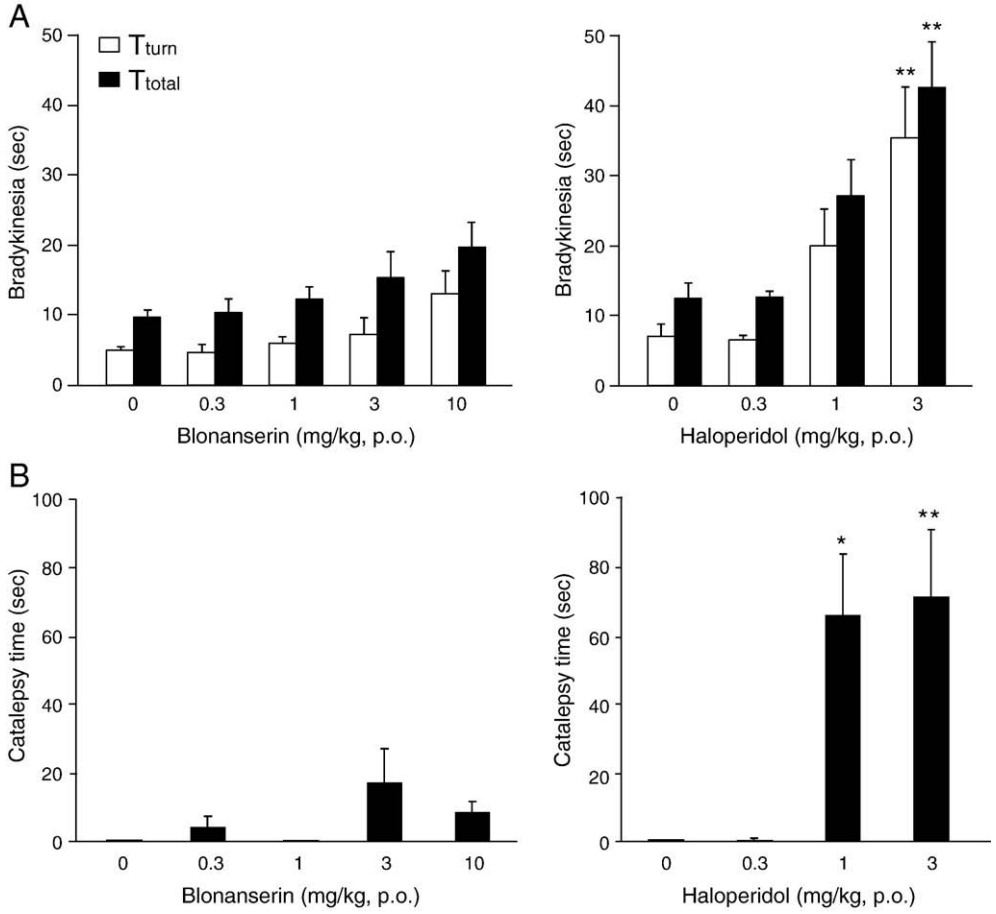

Fig. 2. Effects of blonanserin on T_{turn} and T_{total} values in the pole test (A) and catalepsy time (B) in mice. Blonanserin (0.3 to 10 mg/kg, p.o.) or haloperidol (0.3 to 3 mg/kg, p.o.) was given to mice 30 min before the pole test or catalepsy test. Each column represents the mean ± S.E.M. of 6–7 mice. Statistical differences were determined by Kruskal–Wallis test followed by a Steel–Dwass *post hoc* test. $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the value for the control value with the vehicle alone.

2.4. Fos immunohistochemistry

Two hours after the injection of blonanserin (0.3–10 mg/kg), haloperidol (0.3–3 mg/kg) or a vehicle (Control), the animals were deeply anesthetized with pentobarbital (80 mg/kg, i.p.), perfused transcardially with ice-cold phosphate-buffered saline (PBS) and then with 4% formaldehyde solution. The brain was then removed from the skull and placed in fresh fixative for at least 24 h. After postfixation, coronal sections (30 μm thickness) were cut from each brain using a Microslicer (DSK-1000, Kyoto, Japan).

The staining of Fos-immunoreactivity (IR) was performed by a method published previously (Ohno et al., 2008b, 2009). Briefly, slices were washed with PBS containing 0.3% Triton X-100, and incubated for 2 h in the presence of 2% normal rabbit serum, and then again in the presence of 2% normal rabbit serum and goat c-Fos antiserum (diluted 1:4000, Santa Cruz Biotechnology, sc-52-G) for an additional 18–36 h. The sections were then incubated with a biotinylated rabbit anti-goat IgG secondary antibody (Vector Laboratories, diluted 1:1000) for 2 h. After inactivation of the endogenous peroxidase in the presence of 0.3% hydrogen peroxide for 30 min, the sections were incubated for 2 h with an avidin-biotinylated horseradish peroxidase complex (Vectastain ABC Kit). Fos-IR was then visualized by the diaminobenzidine–nickel staining method.

Fos expression was quantified by counting the number of Fos-IR positive nuclei in the medial prefrontal cortex (mPFC), shell (AcS) and core (AcC) regions of the nucleus accumbens, dorsolateral striatum (dlST) and lateral septum (LS) (Franklin and Paxinos, 2008). The counting of Fos-IR positive nuclei was performed within a $250 \times 250\ \mu m^2$ grid laid over each of the above brain regions by observers who were blinded regarding the animal treatment. The atypical index in Fos expression, based on the difference in numbers of Fos-IR positive cells between the AcS and dlST, was also calculated as described previously (Robertson et al., 1994; Ishibashi et al., 1999). Namely, in each animal treated with blonanserin or haloperidol, the numbers of Fos-IR positive cells in the AcS and dlST were first subtracted by the mean number of the respective control group to normalize the effects of vehicle treatment, and then the normalized number of Fos-IR positive cells in the dlST was subtracted from that in the AcS.

2.5. Drugs

Blonanserin and AD-6048 maleate (Fig. 1) were generous gifts from Dainippon Sumitomo Pharma (Osaka, Japan). Haloperidol was purchased from Sigma-Aldrich (St. Louis, MO). Vectastain ABC and DAB substrate kits were purchased from Vector Laboratories (Burlingame, CA). All other reagents were obtained from commercial sources. Haloperidol, blonanserin and AD-6048 were first dissolved in 1% lactate solution and diluted with saline, and the pH of the solution was adjusted to about 4 to 5 by adding a small amount of 0.5 N NaOH. Control animals were given the same volume of each vehicle solution.

2.6. Statistical analysis

Data are expressed as the mean ± S.E.M. Statistical significance among differences in catalepsy time, T_{turn} and T_{total} values was determined by a Kruskal–Wallis test followed by a Steel–Dwass *post hoc* multiple comparison test. For the analysis of Fos expression, one-way ANOVA followed by a Tukey *post hoc* test was used. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of blonanserin on bradykinesia and catalepsy induction

Oral administration of blonanserin (0.3–10 mg/kg) did not significantly affect either T_{trun} or T_{total} values in the pole test while higher doses (3 and 10 mg/kg) slightly delayed the pole-descending behavior of mice (Fig. 2). Similarly, blonanserin did not increase the catalepsy time at any dose tested. Slight increases in catalepsy time were found with 3 or 10 mg/kg blonanserin, but these changes were not statistically significant. In contrast, haloperidol (0.3–3 mg/kg, p.o.) significantly increased T_{trun} and T_{total} values in a dose-dependent manner (Fig. 2). In addition, marked increases in catalepsy time were obtained with haloperidol. The minimum effective doses of haloperidol for bradykinesia and catalepsy induction were 3 and 1 mg/kg, respectively.

3.2. Effects of blonanserin on forebrain Fos expression

The control animals treated with the vehicle alone showed low levels of Fos expression in the AcS, AcC, mPFC and LS (i.e., mPFC, AcS or AcC: ca. 10 cells/grid, LS: ca. 20 cells/grid), and only a negligible level in the dlST. Oral administration of blonanserin (0.3–10 mg/kg) markedly enhanced Fos expression in the AcS and dlST (Figs. 3 and 4). A significant increase in Fos-IR cells was observed in the AcC, but the LS and mPFC

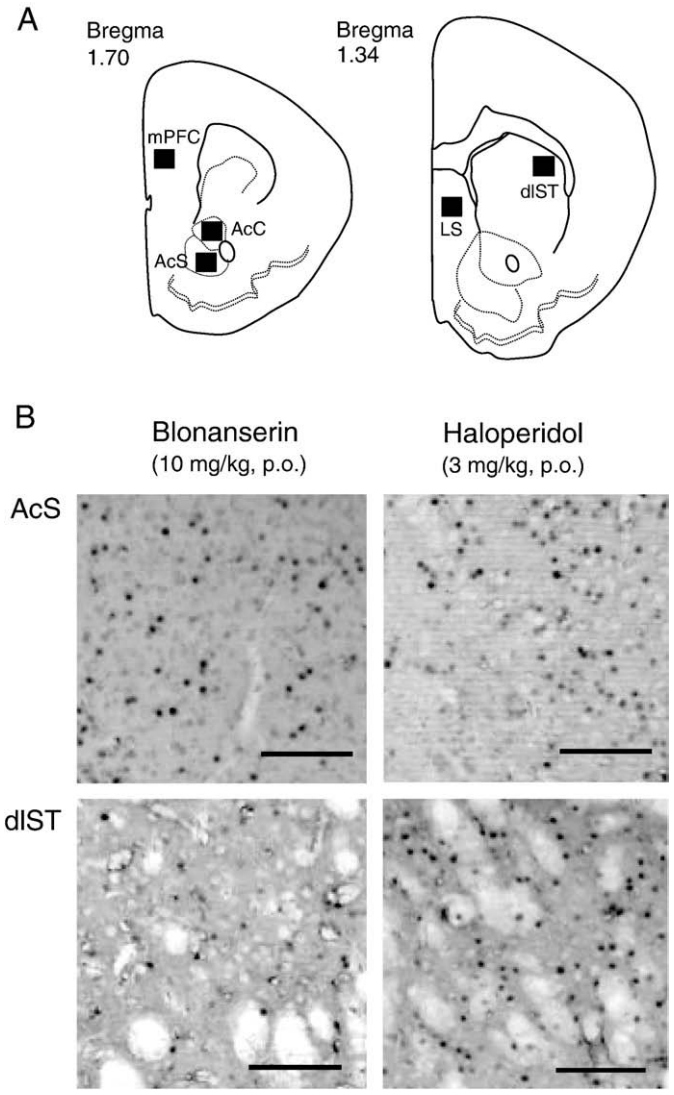

Fig. 3. Blonanserin- and haloperidol-induced Fos expression in mice. A: Schematic illustration of the forebrain areas selected for quantitative analysis of Fos-IR positive cells. Filled boxes indicate the sample areas in the mPFC, AcS, AcC, dlST, and LS. B: Representative photographs illustrating the Fos-IR positive cells in the AcS and dlST of mice treated with blonanserin (10 mg/kg, p.o., left panels) or haloperidol (3 mg.kg, p.o., right panels). Scale bar: 100 μm.

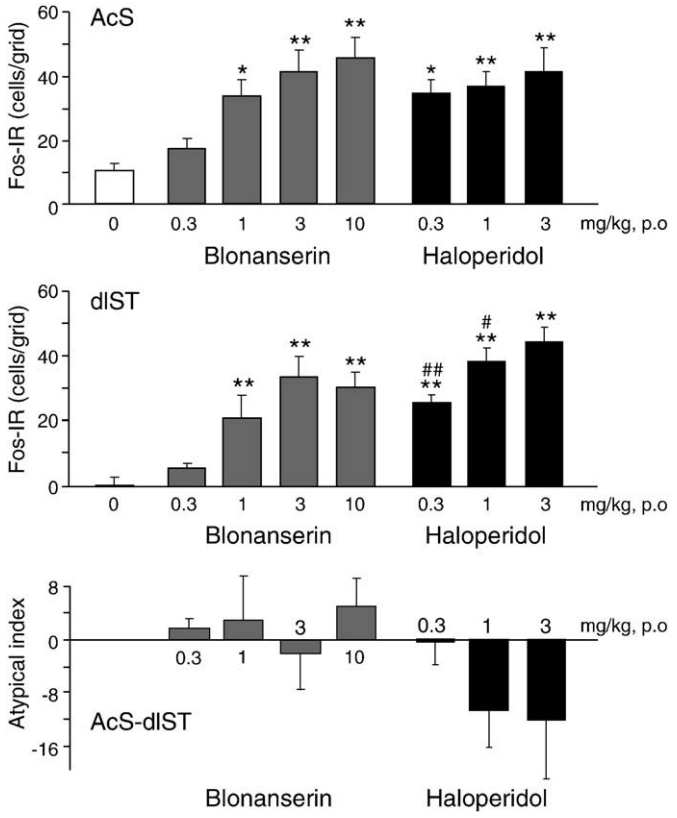

Fig. 4. Effects of blonanserin and haloperidol on Fos expression in the AcS and dlST of mice. Blonanserin (0.3 to 10 mg/kg) and haloperidol (0.3 to 3 mg/kg) were orally given to mice and the brain was removed 2 h after each drug administration. Each column represents the mean ± S.E.M. of 5–7 mice. Atypical index illustrating the difference in numbers of Fos-IR positive cells between the AcS and dlST (AcS − dlST) was calculated as described in Materials and methods. Statistical differences were determined by one-way ANOVA followed by Tukey *post hoc* test (*F*-values of ANOVA: 4.563 for AcS, 17.52 for dlST, and 1.216 for AcS-dlST). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control value with the vehicle alone. $^{\#}P<0.05$, $^{\#\#}P<0.01$; significantly different from the value with the same dose of blonanserin.

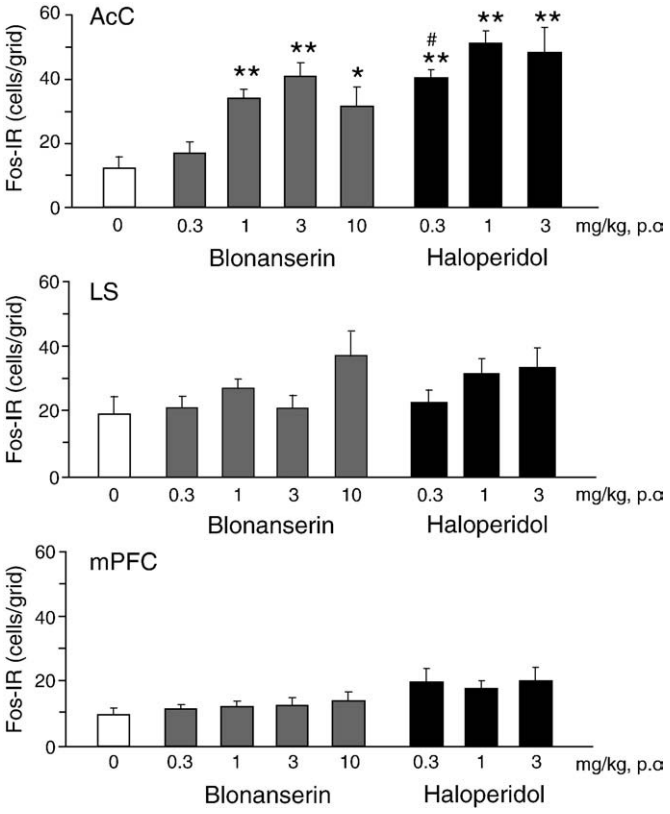

Fig. 5. Effects of blonanserin and haloperidol on Fos expression in the AcC, mPFC and LS of mice. Blonanserin (0.3 to 10 mg/kg) and haloperidol (0.3 to 3 mg/kg) were orally given to mice and the brain was removed 2 h after each drug administration. Each column represents the mean ± S.E.M. of 5–7 mice. Statistical differences were determined by one-way ANOVA followed by Tukey *post hoc* test (*F*-values of ANOVA: 8.396 for AcC, 1.885 for LS, and 1.8736 for mPFC). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control value with the vehicle alone. $^{\#}P<0.05$; significantly different from the value with the same dose of blonanserin.

showed a slight or negligible increase in Fos-IR cells, respectively (Fig. 5). Increases in Fos expression by 10 mg/kg blonanserin, after normalization with the vehicle response, were in the following order, AcS>dlST>AcC>LS>mPFC. In addition, blonanserin-induced Fos expression in the dlST and AcC tended to show a plateau (about 30 cells/grid) at doses of 3 mg/kg (p.o.) or more (Figs. 4 and 5).

Oral administration of haloperidol (0.3–10 mg/kg) markedly increased Fos-IR positive cells in the AcS, AcC and dlST, and weakly in the LS (Figs. 3–5). Intensities in the haloperidol (3 mg/kg, p.o.)-induced Fos expression were in the following order, dlST≥AcC>AcS>LS>mPFC. Thus, Fos expression with moderate doses (0.3 and 1 mg/kg, p.o.) of haloperidol in the dlST was significantly greater than those of blonanserin. In addition, the number of Fos-IR positive cells (ca. 45 cells/grid) in the dlST by 3 mg/kg (p.o.) haloperidol was higher than those (~30 cells/grid) with blonanserin (3 to 10 mg/kg, p.o.). We also compared the atypical indices in Fos expression between blonanserin and haloperidol. Although the differences are not statistically significant, blonanserin contrasted haloperidol by exhibiting higher atypical indices while haloperidol showed negative (dlST-preferential) atypical indices (Fig. 4).

3.3. Effects of AD-6048 on haloperidol-induced catalepsy

Effects of AD-6048, a major metabolite of blonanserin, on haloperidol-induced bradykinesia and catalepsy were examined to assess its potential role in modulating EPS. The animals were first treated with 0.1–3 mg/kg (s.c.) of AD-6048, and 15 min later, haloperidol (0.5 mg/kg, i.p.) was injected. Under these conditions, pretreatment of the animals with AD-6048 dose-dependently attenuated haloperidol-induced bradykinesia in the pole test (Fig. 6). Catalepsy time increased by haloperidol was also reduced by pretreatment with AD-6048 in a dose-dependent manner (Fig. 6).

4. Discussion

In the present study, orally administered blonanserin did not significantly increase T_{turn} or T_{total} values in the pole test at doses up to 10 mg/kg. The actions of blonanserin in the pole test were consistent with those in the catalepsy test, indicating that blonanserin is much weaker than haloperidol (MED = 1–3 mg/kg, p.o.) in inducing EPS. A previous study (Oka et al., 1993) demonstrated that blonanserin and haloperidol antagonized methamphetamine-induced hyperactivity in mice with ED_{50} values of 0.67 and 0.27 mg/kg (p.o.), respectively. Blonanserin also inhibited phencyclidine-induced hyperactivity with similar potency to that of haloperidol (MED = 0.1 mg/kg, p.o., for both drugs). Thus, blonanserin seems to have atypical antipsychotic properties with higher safety margin between antipsychotic actions and EPS induction. In addition, blonanserin, likely with other atypical antipsychotics (e.g., clozapine and olanzapine), also alleviate PCP-induced immobility in the forced swim test, an animal model of negative symptoms (Noda et al., 2000; Nagai et al., 2003).

The atypical features of blonanserin with fewer EPS were further supported by the expression analysis of Fos protein in the forebrain. It

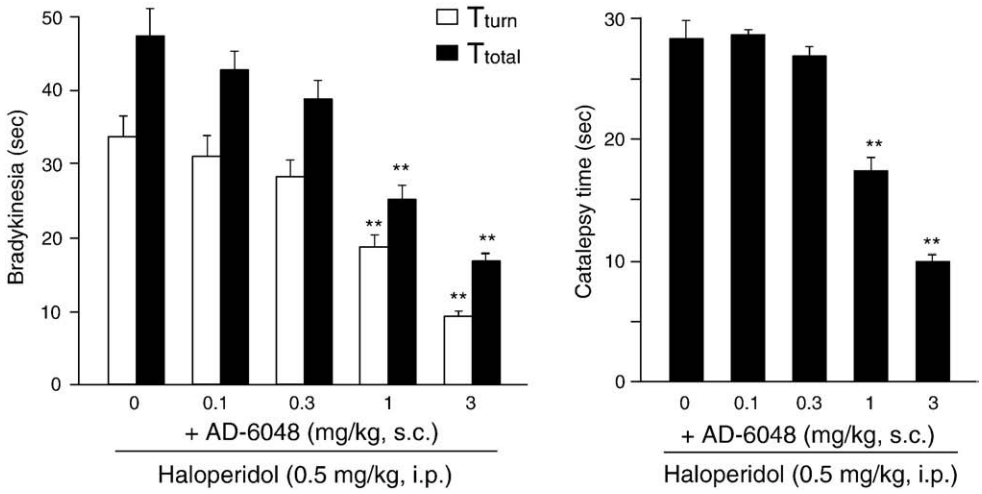

Fig. 6. Effects of AD-6048 on haloperidol-induced bradykinesia and catalepsy in mice. AD-6048 (0.1 to 3 mg/kg, s.c.) was first given to mice and, 15 min later, haloperidol (0.5 mg/kg, i.p.) was injected. The pole test or catalepsy test was performed 30 min after the haloperidol injection. Each column represents the mean ± S.E.M. of 11–14 mice. Statistical differences were determined by Kruskal–Wallis test followed by a Steel–Dwass *post hoc* test. $^{**}P<0.01$; significantly different from the value for the control value with haloperidol alone.

is well documented that endogenous dopamine tonically suppresses Fos expression in striato-pallidal neurons via stimulating G_i-coupled D_2 receptors, and the D_2 antagonists enhance Fos expression through relief of this D_2-mediated negative regulation (Robertson and Fibiger, 1992; Robertson et al., 1994; Marchant and Dorsa, 1993; Chartoff et al., 1999). Furthermore, typical and atypical APDs show a distinct anatomical pattern in inducing Fos expression, in that the typical antipsychotics cause more prominent Fos expression in the dlST, while the atypical ones do so in the AcS (Robertson and Fibiger, 1992; Robertson et al., 1994; Ishibashi et al., 1996, 1999; Oka et al., 2004). In the present study, blonanserin induced marked Fos expression both in AcS and dlST, but its actions in the dlST seemed to be weaker than those of haloperidol. Indeed, the atypical index revealed more preferential actions of blonanserin in the AcS than those of haloperidol. The present study therefore suggests that blonanserin behaves as an atypical antipsychotic agent both in inducing EPS signs and in stimulating forebrain Fos expression. Our results are consistent with a recent clinical report (Garcia et al., 2009) that showed a lower incidence of EPS with blonanserin (10 mg: 26.6%) than with haloperidol (10 mg: 53.3%) during schizophrenia treatment.

A range of values in the atypical indices obtained in the present study was relatively smaller as compared with the original report by Robertson et al. (1994), where haloperidol yielded atypical values of −40 to −60. Although exact reasons for this difference remain uncertain, it might result from species differences (i.e., mice and rats) and/or administration route (i.e., oral and subcutaneous administration).

The mechanisms underlying the atypicality of blonanserin remain uncertain. Blonanserin possesses high affinity to 5-HT_2 receptors and potently inhibits 5-HT_2 receptor-mediated behaviors (e.g., 5-HT agonist-induced head twitches) (Oka et al., 1993; Une and Kurumiya, 2007). Numerous studies have shown that blockade of the 5-HT_2 receptor counteracts D_2 blocking actions of antipsychotics in the striatum (e.g., c-fos mRNA expression, enhanced acetylcholine release or dopamine turnover, and D_2 sensitization following chronic treatment) and attenuates EPS (Saller et al., 1990; Ohno et al., 1994, 1995a,b; Ishibashi et al., 1996; Ishida et al., 1996; Kapur and Remington, 1996; Meltzer et al., 2003). Specifically, the 5-HT_2 antagonists ritanserin and ketanserin inhibit the enhancement of striatal Fos expression by haloperidol. These agents could reduce haloperidol-induced EPS signs (e.g., catalepsy and bradykinesia), but not those induced by the combined 5-HT_2 and D_2 antagonists (e.g., perospirone). Thus, it seems conceivable that 5-HT_2 blocking action of blonanserin at least partly contributes to its low EPS liability. Nonetheless, blonanserin may have other mechanisms than 5-HT_2 antagonism, since it is distinct from other atypical 5-HT_2 and D_2 antagonists in that it exhibits relatively lower affinity to 5-HT_2 receptors (Ki = 0.812 nM) than to D_2 receptors (Ki = 0.142 nM) (Une and Kurumiya, 2007). Although other pharmacological actions such as antagonism at muscarinic acetylcholine receptors (e.g., clozapine and quetiapine) or agonism at 5-HT_{1A} receptors (e.g., perospirone and ziprasidone) have also been implicated in the atypical features of several antipsychotics (Millan, 2000; Raedler et al., 2003; Ishibashi and Ohno, 2004), blonanserin lacks affinity to these receptors.

Blonanserin has a unique metabolite, AD-6048, that has relatively high affinity to D_3 receptors. In a human gene expression system, AD-6048 shows the highest affinity to D_3 receptors (Ki = 0.232 nM), which is 6 times its D_2 affinity (Ki = 1.38 nM), while blonanserin preferentially interacts with D_2 receptors (Ki = 0.142 and 0.494 nM for D_2 and D_3 receptors, respectively). The affinities of AD-6048 to other receptors are very low, except that for 5-HT_{2A} (Ki = 1.28 nM) or 5-HT_6 (Ki = 5.03 nM) (quoted from the NDA file for blonanserin from the Pharmaceuticals and Medical Devices Agency, Japan). We therefore evaluated the effects of AD-6048 on haloperidol-induced bradykinesia and catalepsy and found that it effectively alleviated both EPS signs. Since selective D_3 antagonists attenuate antipsychotics-induced EPS and D_3 preferential antipsychotics show reduced EPS liability (Millan et al., 1997; Perrault et al., 1997; Gyertyán and Sághy, 2007; Gyertyán et al., 2008), it seems likely that interaction of AD-6048 with D_3 receptors plays a role in reducing the EPS of blonanserin. In addition, our results strongly suggest that AD-6048 contributes at least partly to the atypical nature of blonanserin with low EPS liability. Further studies are required to delineate the mechanism of AD-6048 underlying its contribution to the atypical properties of blonanserin.

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