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ORIGINAL INVESTIGATION

Effect of tandospirone, a serotonin-1A receptor partial agonist, on information processing and locomotion in dizocilpine-treated rats

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Abstract
Rationale Augmentation therapy with serotonin-1A receptor (5-HT1A) partial agonists has been suggested to ameliorate psychotic symptoms in patients with schizophrenia.
Objective and methods The objective of the present study was to examine the effect of repeated administration of tandospirone (0.05 and 5 mg/kg) on locomotor activity in a novel environment and on sensorimotor gating in rats treated with the N-*methyl*-D-aspartate receptor antagonist MK-801, which has been used in animal models of schizophrenia. Furthermore, we sought to determine whether the effect of tandospirone on these behavioural measures is blocked by WAY 100635 (0.3 mg/kg), a 5-HT1A receptor antagonist, and whether there is an interaction between haloperidol (0.1 mg/kg; a dopamine-D2 receptor antagonist) and tandospirone.
Results Tandospirone at 5 mg/kg, but not 0.05 mg/kg, decreased locomotor activity in saline or MK-801-treated rats, which were not affected by co-treatment with WAY 100635. Haloperidol decreased locomotion both in saline and MK-801-treated animals, and this effect was not evident in the latter group receiving the higher dose of tandospirone. Tandospirone (5 mg/kg)-induced disruption of sensorimotor gating in saline or MK-801-treated animals was reversed by WAY-100635, but not by haloperidol.
Conclusions These findings suggest that behavioural changes induced by tandospirone are not fully blocked by 5-HT1A antagonists and that tandospirone (5 mg/kg) potentiates the effect of MK-801. Overall, these findings point to an interaction between NMDA and 5-HT_{1A} receptors. Part of the effect of tandospirone on locomotor activity may be mediated by the actions of its active metabolites on other neurotransmitter systems.

Keywords Tandospirone · Schizophrenia · NMDA receptor · MK-801 · Locomotion · PPI · Haloperidol

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Introduction

Serotonin (5-HT) receptors have been suggested as providing potential roles in psychosis and cognition via an influence on various neurotransmitter systems. Among the 5-HT receptor subtypes, 5-HT1A receptors exist as autoreceptors on raphe cell bodies and modify endogenous 5-HT synthesis and release (Hjorth and Sharp 1991; Sharp and

Foster 1991), and they also exist as postsynaptic receptors which directly affect the activity of non-serotonergic neurons in a variety of brain areas (Tanaka et al. 1995).

Tandospirone, an azapirone, is a selective 5-HT1A partial agonist and displays approximately 60% of the effect of the full agonist 8-OH-DPAT (Hamik et al. 1990). Anxiolytic properties of tandospirone, as marketed in Japan, have been demonstrated in human and animal studies (Nishikawa et al. 2007; Nishitsuji et al. 2004; Nishitsuji et al. 2006; Sugimoto et al. 1998). Furthermore, Sumiyoshi and colleagues (Bubenikova-Valesova et al. 2007b; Sumiyoshi et al. 2000; Sumiyoshi et al. 2001b; Sumiyoshi et al. 2001a; Sumiyoshi et al. 2007a) conducted a series of studies on the effect of the addition of tandospirone to ongoing treatment with small to moderate doses of antipsychotic drugs on cognitive function in patients with schizophrenia. Specifically, they found that the addition of tandospirone (30 mg/day) to the typical antipsychotic drug regime for 4–6 weeks improved cognitive function in patients with schizophrenia (Bubenikova-Valesova et al. 2007b; Sumiyoshi et al. 2000; Sumiyoshi et al. 2001b; Sumiyoshi et al. 2001a; Sumiyoshi et al. 2007a).

The glutamatergic neurotransmitter system has been suggested as playing an important role in the aetiopathogenesis of schizophrenia, based on findings on various aspects of neural substrates ranging from molecular interactions to the neuronal network in the human brain (Goff and Coyle 2001; Kristiansen et al. 2007). Moreover, administration of non-competitive antagonists of N-*methyl*-D-aspartate (NMDA) glutamate receptors (phencyclidine, ketamine, and MK-801) has been reported to induce behavioural abnormalities related to symptoms of schizophrenia, such as impairment of information processing and attention, as well as hyperlocomotion in response to a novel environment, which are all ameliorated by antipsychotic use (Bubenikova-Valesova et al. 2008a; Amitai et al. 2007; Bubenikova-Valesova et al. 2008a; Bubenikova et al. 2005; Bubenikova-Valesova et al. 2008b).

In this study, we investigated the effect of repeated administration of tandospirone on deficits in sensorimotor gating and locomotion in rats treated with the NMDA receptor antagonist MK-801. Sensorimotor gating was measured by prepulse inhibition (PPI) of the acoustic startle response. This task consisted of a brief presentation of a high intensity sound stimulus to cause a normal startle reflex response. When this stimulus is preceded by a weak, non-startling stimulus (a prepulse), the subsequent startle response is attenuated (Koch 1999). Deficits in sensorimotor gating have been observed in patients with several neuropsychiatric disorders, including schizophrenia and bipolar disorder (Gogos et al. 2009; Swerdlow et al. 2006). Locomotor activity in response to a novel environment after administration of NMDA antagonists has been widely used in modelling the positive symptoms of schizophrenia. Although hyperlocomotion is not entirely dependent on dopaminergic activation, it is blocked by antipsychotics (van den Buuse 2010).

It was reported that haloperidol blocks the effect of 5-HT1A agonists on information processing (van den Buuse and Gogos 2007). In other studies, 5-HT1A agonists were shown to ameliorate haloperidol-induced catalepsy (Ohno et al. 2008; Ohno et al. 2009). To the best of our knowledge, there is little information on whether 5-HT1A partial agonists, such as tandospirone, interact with D2 receptors in a similar way to the full agonists at 5-HT1A receptors or not. There is also a paucity of animal experiments to determine whether the combination of haloperidol with tandospirone shows an antipsychotic-like profile, despite clinical observations indicating an advantage of augmentation therapy with partial 5-HT1A agonists in patients treated with typical antipsychotic drugs, such as haloperidol.

The principal purpose of this study was to test the hypothesis that moderate stimulation of 5-HT1A receptors with or without the influence of antipsychotic drugs would ameliorate sensorimotor deficits in psychosis-like states (Bubenikova-Valesova et al. 2007b; Sumiyoshi et al. 2008). Furthermore, we sought to determine whether the effect of tandospirone on these behavioural measures is blocked by WAY 100635, an antagonist at 5-HT1A receptors, and whether an interaction occurs between tandospirone and the typical antipsychotic drug haloperidol, which is a dopamine-D2 receptor antagonist.

Materials and methods

Drugs

The treatment regimen with MK-801 was based on our previous report (Bubenikova-Valesova et al. 2007b). The N-*methyl*-D-aspartate receptor antagonist MK-801 (Sigma-Aldrich, Czech Republic; Dizocilpine maleate; [5R,10S]-[+]–5-*methyl*-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine), at a dose of 0.1 mg/kg, was dissolved in saline. The rats received an i.p. injection of MK-801 at a volume of 2 ml/kg for 4 days. The last injection was applied i.p. 15 min prior to the tests. Tandospirone (a gift from Dainippon Sumitomo Pharma Co., Ltd., Japan) was dissolved in saline at a volume of 2 ml/kg. The rats received a s.c. injection of tandospirone at 0.05 or 5 mg/kg for 4 days and a s.c. injection 30 min prior to the tests. Haloperidol (Sigma-Aldrich) at 0.1 mg/kg was dissolved in 15 μl of acetic acid and was added to saline at a volume of 2 ml/kg. The haloperidol (s.c.) was adminis-

tered 60 min before the experiments. The WAY 100635 (0.3 mg/kg, s.c.; Sigma-Aldrich, Czech Republic) (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide) was applied 30 min before the experiments. All animals received the same volume of vehicle per 1 kg of body weight. The control animals received the corresponding vehicle.

Animals

Male Wistar rats (200–250 g, Hannover breed, Konárovice, Czech Republic; $n=356$) were used in this study. Two rats per cage were housed in a temperature-controlled room (21–22°C), with a 12:12 h light/dark regime (lights on at 7:00 a.m.) with free access to food (ST-1 diet) and water. Each rat was only tested once. All manipulations were performed according to the Guidelines of the European Union Council (86/609/EU) and followed the instructions of the National Committee for the Care and Use of Laboratory Animals.

Apparatus and behavioural procedures

Locomotor activity in a novel environment

Locomotor activity, expressed as total distance travelled during 30 min in a box (68×68×30 cm) located in a soundproof room, was measured using a video tracking system for automation of the behavioural experiments (Noldus, Netherlands, EthoVision Colour Pro-Version 3.1), as described previously (Bubenikova-Valesova et al. 2007b).

Prepulse inhibition of acoustic startle response

All testing occurred within a startle chamber (SR-LAB, San Diego Instruments, California, USA). The rats were initially tested by a short session (5 min acclimatisation period plus five single stimuli; 120 dB) 2 days before the experiment. Briefly, the acclimatisation period (75 dB) was presented alone for 5 min. After this, the test began with five initial startle stimuli (125 dB) followed by four different trial types presented in a pseudorandom order: (1) single pulse: 125 db broadband burst, 40 ms duration; (2) prepulse: 13 dB, 20 ms duration above the background noise 100 ms before the onset of the pulse alone; (3) prepulse alone: 13 dB, 20 ms duration above the background noise; (4) no stimulus. A total of five presentations for each trial type were given with an inter-stimulus interval varying from 25 to 30 s. The PPI was calculated as the difference between the average values of the single pulse and prepulse-pulse trials and was expressed as a percentage of the PPI $[100-(\text{mean response for prepulse}-\text{pulsetrials}/\text{startle response forsinglepulse trials})\times 100]$. Data from the four single pulse trials at the beginning of the test session were not included in the calculation of the PPI and acoustic startle response values. Animals showing average startle amplitudes lower than 10 mV were removed from the calculation of the PPI and were marked as non-responders (about 3% of the total number). The number of removed animals did not differ between the treatment groups.

Data analysis

Data are presented as means±SEM. Data from the PPI, startle response and open-field locomotion were first evaluated using three-way analysis of variance (ANOVA) with the dose of tandospirone (0, 0.05, 5 mg/kg) as the factor level and the co-treatment agents MK-801 and WAY100635 (or haloperidol) as between-subject factors. Then, separate two-way ANOVAs were conducted for groups with and without MK-801 treatment, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. A separate evaluation of tandospirone and MK-801 treatment was also performed using two-way ANOVA. Statistical significance was accepted at $p<0.05$ in all instances. We used ten animals per group in all experiments except for the WAY 100635/MK-801 group ($n=9$) and the tandospirone 0.05 mg/kg/WAY 100635/MK-801 group ($n=8$) in the open-field locomotion.

Results

Effect of sub-chronic administration of MK-801 and tandospirone on the startle response and PPI

Two-way ANOVA found main effects of tandospirone [$F(2,54)=17.28$, $p<0.0001$] and MK-801 [$F(1,54)=7.14$, $p<0.001$] on PPI, but the interaction between them was not significant $F(2,54)=2.68$, $p<0.0001$]. Tukey's HSD post-hoc test revealed tandospirone at 5 mg/kg decreased PPI both with and without MK-801 co-treatment ($p<0.01$) (Fig. 1a).

Tandospirone [$F(2,54)=8.38$, $p<0.001$], but not MK-801 [$F(1,54)=0.01$, NS], was found to have an effect on startle amplitudes; tandospirone vs. MK-801 interaction was not significant [$F(2,54)=0.33$, NS] (Fig. 1b).

Effect of sub-chronic administration of MK-801 and tandospirone on locomotor activity

As two-way ANOVA demonstrated, locomotor activity was affected by both tandospirone [$F(2,54)=13.88$, $p<0.0001$] and MK-801 [$F(1,54)=4.56$, $p<0.05$]; interactions between

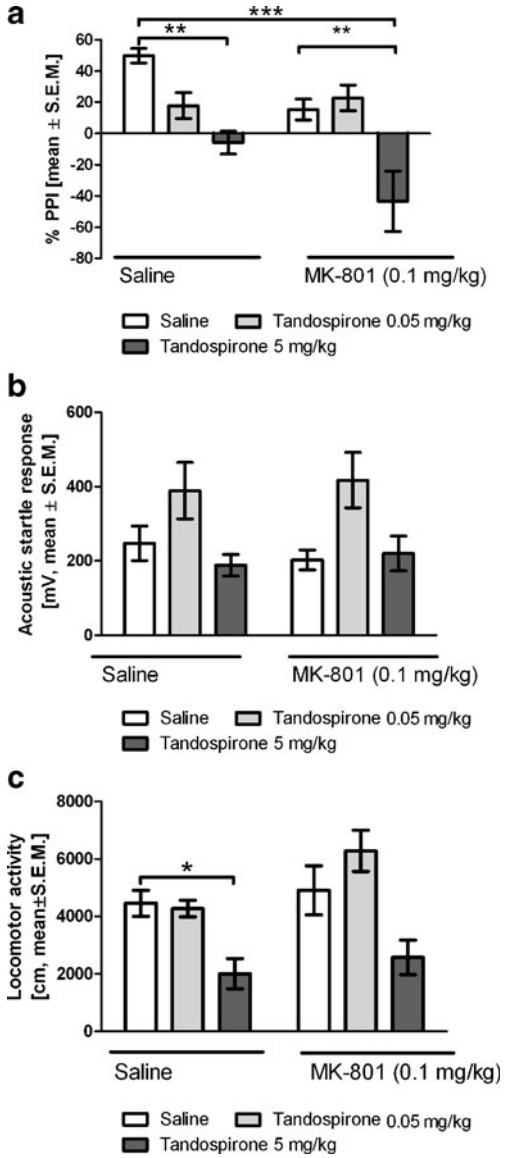

Fig. 1 Effect of sub-chronic administration of tandospirone on schizophrenia-like behaviour induced by MK-801. Tandospirone decreased PPI with and without MK-801 (**a**). The startle response was increased by tandospirone at 0.05 mg/kg in saline and in MK-801-treated rats (**b**). Tandospirone (5 mg/kg) decreased locomotor activity in saline and MK-801-treated rats (**c**). *$p<0.05$; ***$p<0.001$ by Tukey's post-hoc test

the two factors were insignificant [F(2,54)=1.06, NS]. Tukey's HSD post-hoc test found that tandospirone at 5 mg/kg suppressed locomotion ($p<0.05$) (Fig. 1c).

The 5-HT1A receptor antagonist (WAY 100635) blocked the effect of tandospirone on PPI and the startle response with/without MK-801 co-treatment

Three-way ANOVA found significant effects of tandospirone [F(2,107)=18.65, $p<0.0001$], MK-801 [F(1,107)=15.3, $p<0.01$] and WAY 100635 [F(1,107=17.22, $p<0.0001$] on PPI, while tandospirone vs. MK-801 vs. WAY 100635 interaction was not significant [F(2,107)=1.73, NS]. Moreover, there were also significant tandospirone vs. MK-801 [F(2,107=6.09, $p<0.01$] and tandospirone vs. WAY 100635 [F(2, 107=9.31, $p<0.001$], but not MK-801 vs.WAY 100635 interactions.

Subsequent two-way ANOVAs were separately conducted to assess the groups with and without MK-801. The main effects of tandospirone [F(2,53)=23.13, $p<0.0001$] and WAY 100635 [F(1,53)=8.91, $p<0.01$], but not their interaction [F(2,54)=2.32, NS], on PPI were significant among the groups without MK-801. Tukey's HSD post-hoc test revealed a progressive disruption of PPI with increasing dose of tandospirone (0.05 mg/kg, $p<0.05$, compared to the saline/saline group; 5 mg/kg, $p<0.001$). This disruption was partially reversed by WAY 100635 only at 5 mg/kg tandospirone ($p<0.01$, compared with the tandospirone at 5 mg/kg alone group) (Fig. 2a).

Among the MK-801 co-treated groups, tandospirone was found to potentiate MK-801-induced PPI impairment [F(2,54)=7.27, $p<0.01$], while WAY 100635 blocked this effect [F(1,54)=8.95, $p<0.01$]. There was also a significant tandospirone vs. WAY 100635 interaction [F(2,54)=7.12, $p<0.01$]. The post-hoc test revealed that tandospirone potentiated the effect of MK-801 only at 5 mg/kg ($p<0.001$), and that this potentiation was fully blocked by WAY 100635 $p<0.001$) (Fig. 2a).

When analysing data from the startle response, three-way ANOVA did not find the following interactions significant tandospirone vs. MK-801 vs. WAY 100635 [F(2,108)=0.17, NS], tandospirone vs. MK-801 [F(2,108=0.58, NS], and MK-801 vs. WAY 100635 [F(2,108)=0.18, NS], contrasting with significant tandospirone vs. WAY 100635 [F(2,108=6.94, $p<0.01$] interaction. Although ANOVA rejected the main effect of MK-801 [F(1,108)=0.44, NS], it confirmed the effects of tandospirone [F(2,108)=9.35, $p<0.001$] and WAY 100635 [F(2,108)=61.42, $p<0.0001$].

The separate two-way ANOVA tests revealed significant effects of tandospirone [F(2,54)=3.49, $p<0.05$] and WAY 100635 [F(1,54)=33.34, $p<0.0001$], as well as a significant interaction between them [F(2,54)=3.55, $p<0.05$], in rats without MK-801 treatment. Application of 0.5 mg/kg tandospirone moderately, but not significantly, increased the startle response compared to saline/saline rats. This response was significantly reduced by co-treatment with WAY 100635 (Fig. 2b).

The two-way ANOVA test for rats with MK-801 treatment also revealed significant effects of tandospirone [F(2,54)=6.51, $p<0.01$] and WAY 100635 [F(1,54)=28.14, $p<0.0001$], as well as a significant interaction between them [F(2,54)=3.56, $p<0.05$]. In this case, application of 0.5 mg/kg tandospirone led to a significantly stronger startle response compared to

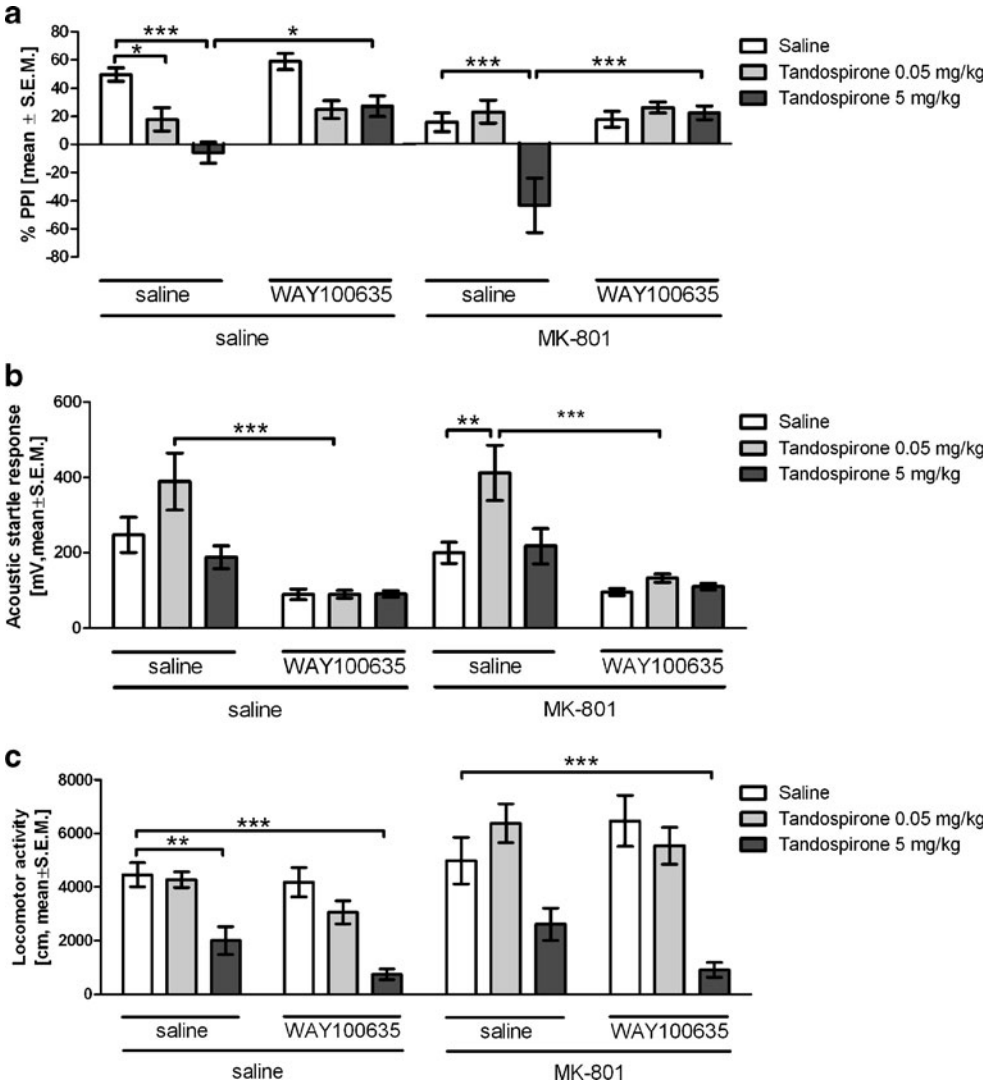

Fig. 2 The effect of WAY 100635 (5-HT1A antagonist) on behaviour induced by tandospirone. WAY 100635 (0.3 mg/kg) blocked the effect of tandospirone (5 mg/kg) with and without MK-801 (**a**). WAY 100635 blocked the effect of tandospirone (0.05 mg/kg) on the startle response with and without MK-801 (**b**). WAY 100635 did not inhibit hypolocomotion induced by tandospirone (5 mg/kg). *$p<0.05$; **$p<0.01$; ***$p<0.001$ by Tukey's post-hoc test

saline/saline rats, which was fully blocked by WAY 100635 (Fig. 2b).

Effect of the 5-HT1A receptor antagonist (WAY 100635) on locomotor activity after co-administration of tandospirone with/without MK-801 co-treatment

Three-way ANOVA conducted on locomotor activity revealed that the following interactions were not significant, tandospirone vs. MK-801 vs. WAY 100635 [F(2,105)= 0.91, NS], tandospirone vs. MK-801 [F(2,105=2.58, NS], and MK-801 vs. WAY 100635 [F(2,105)=0.74, NS]. On the contrary, a significant interaction was found for tandospirone vs. WAY 100635 [F(2,105=3.57, $p<0.05$] interaction as well as main effects of tandospirone [F (2,105)=44.99, $p<0.0001$] and MK-801 [F(1,105)=15.22, $p<0.001$], but not WAY 100635 [F(1,105=3.6, NS].

Subsequent two-way ANOVAs were conducted to assess groups with and without MK-801 separately. Administration of tandospirone decreased locomotion [F(2,54)=26.52, $p<0.0001$)] and WAY 100635 potentiated this effect [F (1,54)=7.04, $p<0.05$; tandospirone vs. WAY 100635 interaction F(2,54)=0.86, NS] in non-MK-801-treated rats. Post-hoc analysis indicated tandospirone (5 mg/kg) alone ($p<0.01$) or in combination with WAY 100635 ($p<0.001$) decreased locomotion compared to the saline/saline group.

Among the MK-801 co-treated groups, two-way ANOVA revealed that tandospirone [F(2,51)=22.1, $p<0.0001$], but not WAY 100635 [F(1,51)=0.35, NS], affected locomotor activity. The interaction between them was not significant [F(2,51)=2.68, NS]. Tukey's HSD post-hoc test revealed that rats treated with MK-801+tandospirone (5 mg/kg)+WAY 100635 ($p<0.01$) walked less than saline/MK-801 controls (Fig. 2c).

Haloperidol had no effect on tandospirone or the startle response and PPI with/without MK-801 co-treatment

Three-way ANOVA on startle response data demonstrated that the following interactions were not significant tandospirone vs. MK-801 vs. haloperidol [F(2,108)=1.02, NS]; tandospirone vs. MK-801 [F(2,108)=1.91, NS]; tandospirone vs haloperidol [F(2,108)=1.02, NS]; and MK-801 vs. haloperidol [F(1,108)=0.99, NS]. Whereas the analysis revealed a significant effect of tandospirone [F(2,108)=11.4, $p<0.0001$], the effects of MK-801 [F(1,108)=1.38,NS] and haloperidol [F(1,108=0.99, NS] remained insignificant.

We examined interactions between tandospirone and haloperidol on PPI in rats with/without MK-801 co-treatment. Three-way ANOVA indicated a significant interaction between tandospirone and haloperidol interaction [F(2,107)=3.18, $p<0.05$] while tandospirone vs. MK-801 vs. haloperidol [F(2,107)=2.94, NS], tandospirone vs. MK-801 [F(2,107)=1.86, NS], and MK-801 vs. haloperidol interactions [F(1,107)=0.003, NS] were not significant. Moreover, there were main effects of tandospirone [F(2,107)=12.0, $p<0.0001$] and MK-801 [F(1,107)=11.72, $p<0.001$], but not haloperidol [F(1,107=0.36, NS].

In the rats that were not given MK-801, two-way ANOVA revealed a significant effect of tandospirone [F(2,54)=16,24, $p<0.001$] but not haloperidol [F(1,54)=0.38, NS] and the interaction between these two was also not significant [F(2,54)=1.05, NS]. Tukey's HSD post-hoc test revealed that tandospirone at 5 mg/kg reduced PPI with ($p<0.01$) and without ($p<0.001$) co-treatment with haloperidol (Fig. 3a).

The interaction between tandospirone and haloperidol on PPI was significant in MK-801-treated rats [F(2,53)=3.79, $p<0.05$]. Tandospirone at a dose of 5 mg/kg worsened MK-801-induced PPI impairment ($p<0.05$), and haloperidol completely blocked tandospirone/MK-801-induced PPI disruption ($p<0.05$). There was also a significant main effect of tandospirone [F(2,53)=3.18, $p<0.05$], but not haloperidol [F(1,53)=0.10, NS] on PPI (Fig. 3a).

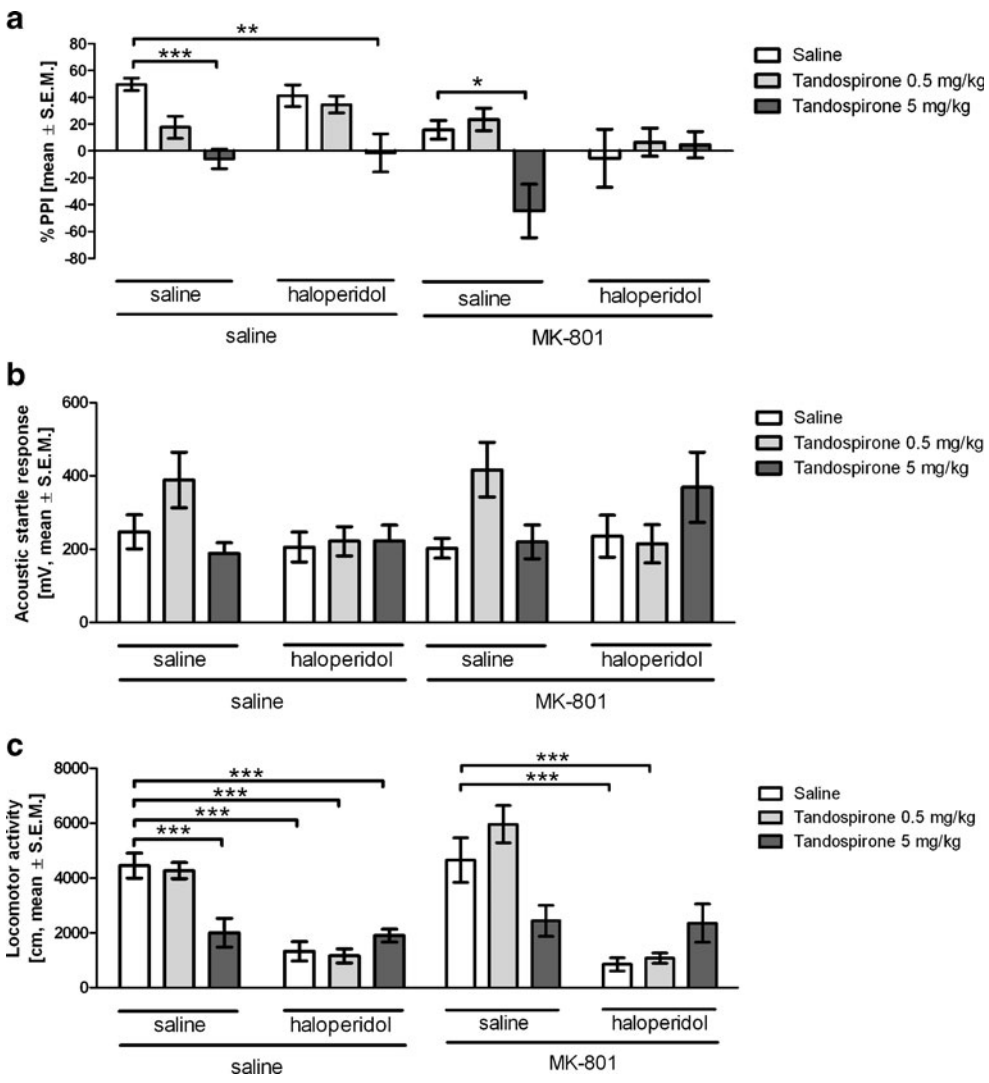

Fig. 3 The effect of haloperidol (D2 antagonist) on behaviour induced by tandospirone. Haloperidol disrupted PPI. Haloperidol did not block the disruption in PPI by tandospirone/MK-801 (**a**). Haloperidol did not change the startle response in combination with tandospirone (**b**). Haloperidol worsened hypolocomotion induced by tandospirone (0.05 mg/kg), but not at a dose of 5 mg/kg

Neither tandospirone [F(2,54)=2.41, NS] nor haloperidol [F(1,54)=2.20, NS] treatment yielded any effect in rats without MK-801 co-treatment. Moreover, the interaction between tandospirone and haloperidol interaction was not significant [F(2,54)=2.26, NS]. Likewise, tandospirone [F(2,54)=1.33, NS] and haloperidol [F(1,54)=0.02, NS] did not show a significant effect on the startle response in rats co-treated with MK-801. Although the interaction between tandospirone and haloperidol was significant [F(2,54)=4.08, $p<0.05$], Tukey's HSD post-hoc test did not identify any difference between the groups (Fig. 3b).

Effect of haloperidol on locomotor activity after co-administration of tandospirone with/without MK-801 treatment

When analysing locomotor activity in the open field, three-way ANOVA did not identify any significant interactions between tandospirone vs. MK-801 vs. haloperidol [F(2,107)=1.06, NS], tandospirone vs. MK-801 [F(2,107)=0.98, NS], or MK-801 vs. haloperidol [F(1,107)=2.81, NS]. However, the interaction between tandospirone and haloperidol interaction was significant [F(2,107)=18.93, $p<0.0001$]. Furthermore, main effects of haloperidol [F(1,107)=81.01, $p<0.0001$] and tandospirone [F(2,107)=3.89, $p<0.05$], but not MK-801 [F(1,107)=3.33, NS], were significant.

Subsequent two-way ANOVA revealed significant effects of tandospirone [F(2,54)=3,68, $p<0.05$] and haloperidol [F(1,54)=49,86, $p<0.0001$], and a significant interaction between these [F(2,54)=11,27, $p<0.0001$] on locomotion in rats without MK-801. Tukey's HSD post-hoc test found that tandospirone at a dose of 5 mg/kg decreased locomotor activity compared with the controls ($p<0.001$). Haloperidol by itself decreased locomotor activity compared with the saline control treatment ($p<0.001$). Administration of haloperidol also decreased locomotion in rats treated with tandospirone at both 0.05 mg/kg ($p<0.001$) and 5 mg/kg ($p<0.001$) (Fig. 3c).

There was significant interaction between tandospirone and haloperidol in MK-801-treated animals [F(2,53)=9.37, $p<0.001$]. Administration of haloperidol significantly decreased locomotor activity in MK-801-treated rats [F(1,53)=38.54, $p<0.001$]. Tukey's HSD post-hoc test showed that haloperidol decreased locomotor activity in tandospirone (0.05 mg/kg)/MK-801-treated rats ($p<0.001$), but not in tandospirone-treated rats (5 mg/kg). There was no main effect of tandospirone treatment [F(2,53)=1.97, NS] (Fig. 3c).

Discussion

Repeated administration of tandospirone at 0.05 or 5.0 mg/kg decreased PPI in rats that were not treated with MK-801 (Fig. 1a). To the best of our knowledge, the effect of tandospirone, a selective 5-HT1A partial agonist, on PPI has not been reported in either animals or humans. Stimulation of 5-HT1A receptors by 8-OH-DPAT (full agonist) or buspirone (partial agonist) decreases PPI (Bubenikova-Valesova et al. 2007b; van den Buuse and Gogos 2007). The effect of tandospirone at 5 mg/kg on PPI was prevented by the acute administration of WAY 100635, a selective 5-HT1A antagonist (Fornal et al. 1996), suggesting that tandospirone decreased PPI via selectively by acting on 5-HT1A receptors. Repeated administration of 5-HT1A receptor agonists desensitises 5-HT1A autoreceptors in the raphe nucleus, but not the postsynaptic receptors in projection areas (Hensler 2003). Therefore, it is possible that tandospirone elicited its effects on PPI via the postsynaptic 5-HT1A receptors, which were blocked by WAY 100635 (Fig. 2a). Interestingly, tandospirone at a dose of 0.05 mg/kg in repeated, but not in acute, administration (data not shown) induced PPI deficits, which were not affected by WAY 100635.

The level of the startle response indicates emotions and attention in rodents. A high startle response disrupts sensory and cognitive processing and is followed by an increase in the heart rate and the activation of other sympathetic systems (Yeomans et al. 2002). Tandospirone at a dose of 0.5 mg/kg increased the acoustic startle response, which was blocked by WAY 100635. An increase in the acoustic startle response was not evident at 5 mg/kg. We suggest that the increase in the startle response by tandospirone could influence the level of PPI.

Locomotor activity in the novel environment was decreased by tandospirone (5 mg/kg; Fig. 1c), which is in accordance with a report by an independent group of investigators (Miller et al. 1992) as well as with our previous findings using another 5-HT1A partial agonist buspirone (Ahlenius et al. 1993; Bubenikova-Valesova et al. 2007a; Haller et al. 2001). However, this is opposite to the response with the full agonist 8-OH-DPAT, which increased locomotor activity (Bubenikova-Valesova et al. 2007a), an effect that was suggested as being mediated by stimulation of the postsynaptic 5-HT1A receptors (Mignon and Wolf 2002). The inhibitory effect of buspirone on locomotion could be explained by its antagonist actions at D2 receptors (McMillen et al. 1983; McMillen and McDonald 1983), while tandospirone has little affinity for these receptors (Hamik et al. 1990).

The inhibitory effect of tandospirone on locomotion was potentiated by the acute administration of WAY 100635 (Fig. 2c). It is assumed that the effects of tandospirone or its metabolites on other neurotransmitter systems are responsible for the ability of this compound to decrease locomotor activity. Tandospirone and buspirone are metabolised to 1-(2-pyrimidinyl)-piperazine (1-PP) in rodents and humans

(Caccia et al. 1982; Miller et al. 1992). It has been shown that 1-PP acts as an antagonist at alpha(2A)-adrenoceptors, and that its administration to mice and rats produces hypolocomotion (Newman-Tancredi et al. 1998; Tatarczynska et al. 1989). It is therefore possible that repeated administration of tandospirone inhibits locomotion via a blockade of alpha(2A)-adrenoceptors by its metabolites. There is little information about the effect of 1-PP on PPI; however, yohimbine, an alpha(2A)-adrenoceptor antagonist, disrupts PPI (Powell et al. 2005). These considerations might not be relevant to the results presented here, which indicates that the effect of tandospirone on PPI is mediated by 5-HT1A receptors.

Repeated administration of MK-801-induced behaviours related to schizophrenia, such as deficits in sensorimotor gating, as measured by PPI (Bubenikova-Valesova et al. 2008a). In our study, we used the sub-chronic (4 days) administration of MK-801 and tandospirone. Based on our observations (not shown), we assumed that the 4-day application in our experimental paradigm changed the behaviour and expression of NMDA receptors in a way that mimicked chronic treatment. Antipsychotic agents are expected to reverse the effect of MK-801 while the high-dose tandosprione (5 mg/kg) exacerbated the MK-801-induced PPI deficits, an effect blocked by WAY 100635 (Fig. 2a). Acute administration of MK-801 (0.1 mg/kg) has been shown to enhance locomotion (Bubenikova-Valesova et al. 2008a), and this has been used as a model of the positive symptoms of schizophrenia (van den Buuse 2010); however, repeated administration of the same dose of MK-801 has been shown to induce tolerance and it did not increase locomotor activity (Amitai et al. 2007). Furthermore, tandospirone, at 5 mg/kg, inhibited locomotor activity in MK-801-treated rats, which was not affected by WAY 100635 (Fig. 2c).

Tandospirone decreased locomotor activity in MK-801-treated rats, but exacerbated MK-801-induced PPI disruption (Fig. 1a, c). Clinical trials reporting a beneficial influence of tandospirone on cognition were based on data from schizophrenia patients treated with typical antipsychotic drugs, such as haloperidol, which are D2 antagonists (Sumiyoshi et al. 2000; Sumiyoshi et al. 2001b; Sumiyoshi et al. 2001a). Therefore, we investigated the effect of co-administration of haloperidol on behavioural changes induced by the repeated administration of tandospirone with and without co-treatment by MK-801. Acute administration of haloperidol did not influence PPI or the effect of tandospirone on PPI in rats without MK-801 treatment (Fig. 3a). In accordance to findings in the literature, we found that haloperidol did not block PPI disruption induced by a 5-HT1A agonist (van den Buuse and Gogos 2007). A low dose (0.1 mg/kg) of haloperidol decreased locomotion by itself or when combined with a low, but not high, dose of tandospirone. It is well documented that 5-HT1A agonists ameliorate haloperidol-induced catalepsy (Ohno et al. 2008; Ohno et al. 2009). This line of evidence is consistent with our observations that a high dose of tandospirone counteracted haloperidol-induced hypolocomotion.

In MK-801-treated rats, haloperidol did not reverse PPI disruption induced by tandospirone (5 mg/kg), similar to the effect of 5-HT1A receptor antagonists. Haloperidol by itself did not influence PPI deficits induced by MK-801, in accordance with the results of our previous study (Bubenikova et al. 2005). On the other hand, haloperidol decreased locomotor activity in MK-801-treated rats, which was blocked by tandospirone (5 m/kg) (Fig. 3c).

Augmentation therapy with 5-HT1A partial agonists, such as tandospirone and buspirone, in patients treated with typical or atypical antipsychotics produces benefits to a range of cognitive functions, e.g. verbal memory, executive function, memory organisation and attention/information processing (Sumiyoshi et al. 2001a; Sumiyoshi et al. 2007a; Sumiyoshi et al. 2007b). Therefore, our initial hypothesis was that tandospirone, with or without co-administration with haloperidol, would show antipsychotic-like effects in MK-801-treated rats.

Our results indicate an interaction between 5-HT1A receptors and NMDA receptors on behavioural levels. Several studies show that activation of 5-HT1A receptors inhibits the function of NMDA receptors on the molecular level, although the mechanisms underlying these interactions are largely unknown (Gu et al. 2007; Yuen et al. 2005). Based on this, 5-HT1A agonists were assumed to inhibit the effect of NMDA antagonists. However, our previous study showed that acute administration of the 5-HT1A full agonist 8-OH-DPAT, at a low-dose, blocked MK-801-induced PPI deficits. In addition, it is possible that 5-HT1A partial agonists, such as tandospirone and buspirone (azapirone derivatives), behave differently from full 5-HT1A agonists. Also, active metabolites of these 5-HT1A partial agonists have an affinity for alpha(2A)-adrenoceptors, as discussed above.

The role of 5-HT1A receptors in the pathophysiology and treatment of schizophrenia is still unresolved. The findings in this study suggest that the mode of action of clinically used azapirone derivatives may not be entirely through 5-HT1A receptors. Investigations of 5-HT1A partial agonism, using agents that do not produce active metabolites which influence other neurotransmitter systems, e.g. the adrenergic system, should be the subject of further studies.

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