Full Paper

Effects of Tansospirone, a 5-HT_{1A} Agonistic Anxiolytic Agent, on Haloperidol-Induced Catalepsy and Forebrain Fos Expression in Mice Yukihiro Ohno^{1,*}, Saki Shimizu¹, and Junta Imaki¹¹Laboratory of Pharmacology, Osaka University of Pharmaceutical Sciences, Takatsuki, Osaka 569-1094, Japan

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Abstract. We studied the effects of tansospirone, a 5-HT_{1A} agonistic anxiolytic agent, on haloperidol-induced catalepsy and forebrain Fos expression in mice. Haloperidol (0.5 mg/kg, i.p.) markedly increased the catalepsy time and enhanced Fos expression in the shell (AcS) and core (AcC) regions of the nucleus accumbens, the dorsolateral striatum (dlST), and the lateral septal nucleus (LSN). Tansospirone (0.1 – 1 mg/kg, s.c.) significantly alleviated haloperidol-induced catalepsy in a dose-dependent manner, which was antagonized by WAY-100135 (a selective 5-HT_{1A} antagonist). The anticataleptic dose of tansospirone (1 mg/kg, s.c.) significantly reduced haloperidol-induced Fos expression in the dlST. This inhibition by tansospirone was regionally specific, and it failed to affect haloperidol-induced Fos expression either in the AcS, AcC, or LSN. In addition, the reversal of haloperidol-induced striatal Fos expression by tansospirone was antagonized by WAY-100135. These results support the notion that stimulation of 5-HT_{1A} receptors region-specifically counteracts the D₂-blocking actions of haloperidol in the striatum, which may account for the ameliorative effects of 5-HT_{1A} agonists on antipsychotic-associated extrapyramidal disorders.

Keywords: extrapyramidal motor disorder, Fos expression, 5-HT_{1A} receptor, tansospirone, antipsychotic

Introduction

Antipsychotic drugs (APDs) often induce extrapyramidal side effects (EPS) in patients with schizophrenia, including akathisia, tremor, hypokinesia, and impaired involuntary movements (e.g., dystonia and dyskinesia). It is now known that the serotonergic system plays an important role in modulating extrapyramidal motor disorders. Specifically, the blockade of 5-HT_{2A} receptors can reduce APD-induced EPS (e.g., catalepsy, bradykinesia, dyskinesia) in various animal models (1 – 3). The EPS-amelioration through 5-HT_{2A}-receptor antagonism underlies the atypical feature of several new generation APDs such as risperidone, olanzapine, and perospirone (3).

A line of studies demonstrated that 5-HT_{1A} agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) significantly inhibit APD-induced EPS (4 – 6) or

motor disabilities in animal models of Parkinson's disease (7). It is therefore conceivable that, besides 5-HT_{2A} antagonism, 5-HT_{1A} agonism also alleviates APD-induced EPS and/or Parkinson's disease (3). Although the mechanisms for these actions of 5-HT_{1A} agonists are yet to be clarified, we have previously shown that alleviation of haloperidol-induced EPS by 8-OH-DPAT was resistant against the serotonergic denervation with *p*-chlorophenylalanine (a 5-HT synthetase inhibitor) (8). Consistent with previous findings (4, 7, 9), our results suggest that 8-OH-DPAT alleviates APD-induced EPS probably through activating postsynaptic 5-HT_{1A} receptors (including heteroreceptors). In addition, anticataleptic 8-OH-DPAT reversed the haloperidol (D₂ antagonism)-induced Fos expression in the dorsolateral striatum (dlST) and the core region of the nucleus accumbens (AcC), without affecting other brain regions examined (10). Thus, it was suggested that stimulation of 5-HT_{1A} receptors could reduce the D₂-blocking actions of APDs specifically in brain regions (i.e., dlST and AcC) that are related to EPS induction (11 – 13). However, studies on the action of 5-HT_{1A} agonists in the

*Corresponding author. yohno@gly.oups.ac.jp

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forebrain Fos expression are still limited, and the action of 5-HT_{1A} agonists and their specificities in modulating APD-induced Fos expression remains to be verified.

Tandospirone is a 5-HT_{1A} agonistic anxiolytic agent that preferentially interacts with 5-HT_{1A} receptors (14). It stimulates human 5-HT_{1A} receptors with high intrinsic activities (i.e., 80%–90% that of 5-HT) (15). In addition, recent studies showed that tandospirone improves EPS signs associated with APD treatments and in some animal models of Parkinson's disease (8, 16, 17). Therefore, to further clarify the action of 5-HT_{1A} agonists and their specificities in modifying APD-induced Fos expression, we studied the effects of tandospirone, a clinically available 5-HT_{1A} agonist, on haloperidol-induced catalepsy and forebrain Fos expression and examined their responses to the 5-HT_{1A} antagonist.

Materials and Methods

Animals

Male ddY mice (Japan SLC, Inc., Shizuoka) 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 experimental protocols of this study were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

Catalepsy test

The catalepsy test was carried out as described previously (10). Briefly, the fore paws of the animals were placed on a horizontal bar positioned at 5 cm above the bench surface, and the time spent for the animals to show the cataleptic posture, which was defined as an immobile posture while keeping both forelimbs on the bar, was measured with a maximum limit of 30 s.

Based on a previously determined dose–response curve for catalepsy induction (10), the dose of haloperidol was set at 0.5 mg/kg (i.p.) and was given to the animals 30 min before the catalepsy test. Tandospirone (0.1–1 mg/kg) was injected subcutaneously 15 min before the haloperidol injection. In the experiments with WAY-100135 (a 5-HT_{1A} antagonist), this agent (10 mg/kg, i.p.) was administered simultaneously with tandospirone 15 min before the haloperidol injection.

Fos immunohistochemistry

The mice were first treated with tandospirone (1 mg/kg, s.c.) or saline and, 15 min later, were given haloperidol (0.5 mg/kg, i.p.) under the same protocol as employed in the catalepsy test. In some animals, WAY-100135 (10 mg/kg, i.p.) was given simultaneously with

tandospirone (1 mg/kg, s.c.) to evaluate the involvement of 5-HT_{1A} receptors in the action of tandospirone in the striatum. For this study, it was confirmed in the preliminary experiments that the vehicle (saline) treatment alone did not affect the inhibitory action of tandospirone on haloperidol Fos expression (data not shown). At 2 h after the haloperidol injection, the animals were deeply anesthetized with pentobarbital (80 mg/kg, i.p.), perfused transcardially with ice-cold phosphate-buffered saline (PBS), and then again with 4% formaldehyde solution. The brain was removed from the skull and placed in fresh fixative for at least 24 h. After postfixation, coronal sections (30- μ m thickness) were cut from each brain using a Microslicer (DTK-3000; Dosaka EM Co., Ltd., Kyoto).

The staining of Fos-immunoreactivity (IR) was performed by the previously described method (10, 18). Briefly, slices were washed with PBS containing 0.3% Triton X-100, 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, CA, USA) for an additional 18–36 h. The sections were then incubated with biotinylated rabbit anti-goat IgG secondary antibody (diluted 1:1000; Vector Laboratories, CA, USA) 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 avidin-biotinylated horseradish peroxidase complex (Vectastain ABC Kit, Vector Laboratories). Fos-IR was then visualized by the diaminobenzidine-nickel staining method.

Fos expression was quantified by counting the number of Fos-IR positive cells in the medial prefrontal

Fig. 1. Schematic drawings of the mouse forebrain areas selected for quantitative analysis of Fos-IR-positive cells. Filled boxes indicate the sample areas (250 × 250 μ m²) in the mPFC, AcS, AcC, dIST, and LSN. cc: corpus callosum, cg: cingulum.

cortex (mPFC), the shell region of the nucleus accumbens (AcS), AcC, dlST, and the lateral septal nucleus (LSN) (Fig. 1). The counting was performed within a $250 \times 250 \mu\text{m}^2$ grid laid over each of the above brain regions by observers who were kept unaware of the animal treatment. The atypical index of haloperidol, based on the difference in the numbers of Fos-IR positive cells between the AcS and dlST, was also calculated as described previously (13, 18).

Drugs

Haloperidol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tandospirone was a gift from Daiippon Sumitomo Pharma Co., Ltd. (Osaka). WAY-100135 hydrochloride was obtained from Tocris (Bristol, UK). Vectastain ABC and DAB substrate kits were purchased from Vector Laboratories (CA, USA). All other reagents were obtained from commercial sources. Haloperidol was 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. Tandospirone and WAY-100135 were dissolved in physiological saline. The control animals were given the same volume of each vehicle solution. All drugs or vehicles were injected intraperitoneally or subcutaneously at a volume of 5 ml/kg.

Statistical analyses

Data were expressed as the mean $\pm$ S.E.M. Statistical significance of the differences in catalepsy time was determined by a non-parametric analysis since some animals showed the upper limit (i.e., 30 s) of the observation for catalepsy. Namely, comparisons among multiple groups (i.e., dose-response for tandospirone) were determined by the Kruskal-Wallis test followed by the Steel-Dwass *post hoc* multiple comparison test. Comparisons between two groups were made by the Mann-Witney test. For the analysis for Fos expression, comparisons were made between two groups using the Student's *t*-test. Differences were considered to be statistically significant for values of $P < 0.05$.

Results

Effects of tandospirone on haloperidol-induced catalepsy

Haloperidol (0.5 mg/kg, i.p.) markedly increased the catalepsy time of mice. Pretreatment of animals with tandospirone (0.1–1 mg/kg, s.c.), 15 min before the haloperidol injection, dose-dependently attenuated the haloperidol-induced catalepsy (Fig. 2A). The reduction of the haloperidol-induced catalepsy by tandospirone

Fig. 2. Effects of tandospirone (TAND) on haloperidol (HAL)-induced catalepsy and its reversal by WAY-100135 (WAY). A: Effects of TAND (0.1–1 mg/kg, s.c.) on HAL (0.5 mg/kg, i.p.)-induced catalepsy. TAND was given to mice 15 min before the HAL injection and the catalepsy test was performed 30 min after the HAL injection. B: Effects of WAY (10 mg/kg, i.p.) on the reversal of HAL (0.5 mg/kg, i.p.)-induced catalepsy by TAND (1 mg/kg, s.c.). WAY was given simultaneously with TAND 15 min before the HAL injection, which was followed by the catalepsy test 30 min later. Each column represents the mean $\pm$ S.E.M. of 9–16 mice. ** $P < 0.01$: Significantly different from the control value with HAL alone. ## $P < 0.01$: Significantly different from the value with HAL plus TAND.

was statistically significant at doses of 0.3 and 1 mg/kg ($P < 0.01$). When the selective 5-HT_{1A} antagonist WAY-100135 (10 mg/kg, i.p.) was simultaneously injected with tandospirone (1 mg/kg, s.c.), the ameliorative action of tandospirone on haloperidol-induced catalepsy was completely antagonized (Fig. 2B).

Effects of tandospirone on haloperidol-induced Fos expression in the striatum

We next examined the effects of the anticataleptic dose (1 mg/kg, s.c.) of tandospirone on haloperidol-

Fig. 3. Effects of tandospirone (TAND) on haloperidol (HAL)-induced Fos expression in the dlST and its reversal by WAY-100135 (WAY). **A:** Photographs illustrating the Fos-IR-positive cells in the dlST of HAL (0.5 mg/kg, i.p.)-treated (top), HAL (0.5 mg/kg, i.p.) + TAND (1 mg/kg, s.c.)-treated (middle), and HAL (0.5 mg/kg, i.p.) + TAND (1 mg/kg, s.c.) + WAY (10 mg/kg, i.p.)-treated mice (bottom). The paradigm of the drug treatments was same as the catalepsy test, and the brain was removed 2 h after the HAL injection. Scale bar: 100 μ m. **B:** Effects of TAND (1 mg/kg, s.c.) on HAL (0.5 mg/kg, i.p.)-induced Fos expression in the dlST and its reversal by WAY (10 mg/kg, i.p.). Each column represents the mean $\pm$ S.E.M. of 4–7 mice. ** P <0.01: Significantly different from the control value with vehicle alone. # P <0.05: Significantly different from the value with HAL alone. † P <0.05: Significantly different from the value with HAL + TAND.

induced Fos expression in the forebrain. The control animals showed only negligible Fos expression in the dlST, and the number of Fos-IR positive cells was markedly increased by haloperidol (Fig. 3). The haloperidol-induced Fos expression was significantly inhibited by tandospirone (P <0.05) (Fig. 3). The number of Fos-IR positive cells in the dlST was about 40 cells/grid with haloperidol (0.5 mg/kg, i.p.) alone, but this value was reduced to about 20 cells/grid following combined treatment with haloperidol and tandospirone (1 mg/kg, s.c.). In addition, WAY-100135 (10 mg/kg, i.p.) significantly antagonized the inhibitory effects of tandospirone on haloperidol-induced Fos expression in the dlST (Fig. 3).

Effects of tandospirone on haloperidol-induced Fos expression in the nucleus accumbens and other areas

Treating the animals with vehicle (Control) increased Fos expression to some extent (ca. 15 cells/grid) in the AcS and AcC (Fig. 4). Haloperidol (0.5 mg/kg, i.p.) markedly enhanced Fos expression both in the AcS and AcC, and the number of Fos-IR-positive cells increased to about 40 cells/grid in both structures. Pretreatment of the animals with tandospirone (1 mg/kg, s.c.) did not significantly change the haloperidol-induced Fos expression in either the AcS or AcC (Fig. 4).

We also estimated the atypical index of haloperidol for Fos expression with or without tandospirone. The increases in the mean number of Fos-positive cells by haloperidol were 23.0 in the AcS and 37.6 in the dlST without tandospirone, but these values became 27.9 and

17.7 with tandospirone, respectively (Fig. 3B and Fig. 4B). Thus the atypical index, defined as the difference in the number of Fos-IR-positive cells between the AcS and the dlST (i.e., AcS minus dlST), was -14.6 (dlST-preferential) with haloperidol alone, but was +10.2 (AcS-preferential) following the combined treatment with haloperidol and tandospirone.

In the LSN and mPFC, control animals showed a weak to moderate Fos expression (ca. 15–20 cells/grid) (Fig. 5). Haloperidol treatment significantly increased the number of Fos-IR-positive cells in the LSN, but not in the mPFC. The treatment with tandospirone (1 mg/kg, s.c.) did not significantly affect the haloperidol-induced Fos expression either in the LSN or in the mPFC (Fig. 5).

Discussion

Consistent with previous studies (9, 12, 18), haloperidol at the cataleptogenic dose markedly enhanced Fos expression in the dlST, AcS, AcC, and LSN without affecting that in the mPFC. It is known that endogenous dopamine tonically suppresses Fos expression in striato-pallidal neurons via stimulating G_i-coupled D₂ receptors, and APDs (i.e., D₂ antagonists) enhance Fos expression through a relief of this D₂-mediated negative regulation (12, 13, 19, 20). APD-induced Fos expression is thought to be dependent on protein kinase A (PK-A) activation since the targeted disruption of the RIIb subunit of PK-A abolished striatal Fos expression (21). In addition, multiple systems including glutamatergic (e.g., NMDA

Fig. 4. Effects of tandospirone (TAND) on haloperidol (HAL)-induced Fos expression in the AcS and AcC. A: Photographs illustrating Fos-IR-positive cells in the AcS and AcC of HAL (0.5 mg/kg, i.p.)-treated (left panels) and HAL (0.5 mg/kg, i.p.) + TAND (1 mg/kg, s.c.)-treated (right panels) mice. The paradigm of the drug treatments was the same as the catalepsy test, and the brain was removed 2 h after the HAL injection. The AcS and AcC in each treatment group were obtained from the same animal. Scale bar: 100 μ m. B: Effects of TAND (1 mg/kg, s.c.) on HAL (0.5 mg/kg, i.p.)-induced Fos expression in the AcS and AcC. Each column represents the mean $\pm$ S.E.M. of 6–8 mice. ** P <0.01: Significantly different from the control value with vehicle alone. n.s.: Not significant.

receptors) and purinergic (e.g., A_2 receptors) systems are known to influence APD-induced striatal Fos expression through modulating Ca^{2+} - and Gs-sensitive adenylate cyclase activity, respectively (20, 22, 23).

The anticataleptic dose of tandospirone significantly attenuated haloperidol-induced Fos expression in the dlST, suggesting that stimulation of 5-HT_{1A} receptors counteracts the D₂-blocking actions of haloperidol in the striatum. This notion was further supported by the fact that reversal of both haloperidol-induced catalepsy and Fos expression by tandospirone was antagonized by

Fig. 5. Effects of tandospirone (TAND; 1 mg/kg, s.c.) on haloperidol (HAL; 0.5 mg/kg, i.p.)-induced Fos expression in the LSN (A) and mPFC (B). The paradigm of the drug treatments was the same as the catalepsy test, and the brain was removed 2 h after the HAL injection. Each column represents the mean $\pm$ S.E.M. of 5–7 mice. * P <0.05: Significantly different from the control value with vehicle alone. n.s.: Not significant.

WAY-100135. The action of tandospirone in forebrain Fos expression was region-specific, and it did not alter haloperidol-induced Fos expression in either the AcS, AcC, LSN, or mPFC. Regional specificity of the action of tandospirone was superior to that of 8-OH-DPAT reported in previous studies (10, 24), where 8-OH-DPAT attenuated haloperidol-induced Fos expression not only in the dlST, but also in the AcC. Tremblay et al. (24) also reported that 8-OH-DPAT enhances haloperidol-induced Fos expression in the mPFC. Thus, the present and previous (10, 24) results indicate that the striatum is the common site of action for 5-HT_{1A} agonists to attenuate both haloperidol-induced Fos expression and catalepsy. Although the reason for the difference in actions between tandospirone and 8-OH-DPAT is presently uncertain, 8-OH-DPAT also possesses a potent agonistic activity at 5-HT₇ receptors (25, 26) and an affinity for α_{2A} receptors (27). These actions of 8-OH-DPAT might be involved in its actions in the AcC

and/or mPFC.

It has been shown that antipsychotic-induced Fos expression in the striatum is closely related to the induction of APD-induced EPS, whereas that in the nucleus accumbens is predictive for antipsychotic effects of APDs (13, 18, 21, 28). Thus, the typical and atypical APDs show distinct anatomical patterns in inducing Fos expression, such that the typical APDs with greater EPS cause more prominent Fos expression in the striatum. Robertson et al. (13) first proposed the atypical index of Fos expression, based on the difference in numbers of Fos-IR-positive cells in the AcS and dLST, which serves as a predictor for distinguishing atypical APDs from typical ones. The typical APDs (e.g., haloperidol and fluphenazine) commonly show negative atypical values (dLST-preferential), whereas the atypical APDs (e.g., clozapine and risperidone) exhibit positive values (AcS-preferential) (13, 18, 21). In the present study, due to the region-specific action of tandospirone, the atypical index of haloperidol shifted from a negative to a positive value with tandospirone. Our results suggest that combined administration of haloperidol with tandospirone allows haloperidol to behave as an atypical APD and that tandospirone may be useful as an adjunct remedy for schizophrenia treatment. Regarding the beneficial effects for schizophrenia, tandospirone is also known to be effective for cognitive deficits in patients with schizophrenia (29, 30).

The action sites and mechanisms of tandospirone for the reversal of APD-induced striatal Fos expression and EPS remain to be clarified. 5-HT_{1A} receptors are G_i-coupled, and their activation by tandospirone cause inhibition of adenylate cyclase (15), which might counteract haloperidol-enhanced PK-A activity and Fos expression within the striatum. On the other hand, several studies (31, 32) have shown that 5-HT_{1A} agonists inhibit the cortico-striatal glutamate pathway and reduce extracellular glutamate levels in the striatum (31, 32). In addition, intracortical perfusion of 5-HT_{1A} agonists also inhibited striatal glutamate release, implying that cortical 5-HT_{1A} receptors regulate activity of the cortico-striatal glutamate pathway (32). Since the inhibition of glutamatergic neurons by NMDA antagonists is known to reduce haloperidol-induced striatal Fos expression and catalepsy induction (20, 22), tandospirone might activate cortical 5-HT_{1A} receptors and counteract the action of haloperidol by inhibiting the cortico-striatal glutamate neurons. Consistent with this hypothesis, our preliminary results revealed that bilateral microinjection (5 µg/0.5 µl per side) of tandospirone into the dLST failed to affect the induction of haloperidol-induced catalepsy under the present experimental conditions. Further microinjection studies examining a multiple

dose range and different injection sites (e.g., cerebral cortex) are required to delineate the precise mechanisms for the EPS-ameliorative actions of 5-HT_{1A} agonists.

In summary, we studied the effects of the 5-HT_{1A} agonistic anxiolytic tandospirone on haloperidol-induced catalepsy and forebrain Fos expression in mice. The present study confirmed that tandospirone significantly ameliorates haloperidol-induced catalepsy by stimulating 5-HT_{1A} receptors. In addition, anticataleptic tandospirone significantly reduced the haloperidol-induced Fos expression region-specifically in the dLST without affecting that in other brain regions examined, and this effect was reversed by the 5-HT_{1A} antagonist. These findings further support the notion that stimulation of 5-HT_{1A} receptors selectively counteracts the D₂ blocking action of haloperidol in the striatum, which can at least partly account for the EPS-ameliorative actions of 5-HT_{1A} agonists.

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