Serotonergic Mechanisms in Anxiolytic Effect of Tansospirone in the Vogel Conflict Test

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ABSTRACT—To clarify which 5-HT_{1A} receptors, autoreceptors located in the raphe nuclei or postsynaptic receptors in the forebrain areas receiving a 5-HT input, mediate the anticonflict action of tansospirone (a 5-HT_{1A} receptor-related anxiolytics), the behavioral effects of tansospirone were studied in 5,7-dihydroxytryptamine (5,7-DHT) treated rats. By measuring both monoamines and their metabolite levels and densities of [³H]8-OH-DPAT binding in 5,7-DHT-treated rat brain, we confirmed that pretreatment with 5,7-DHT destroyed 5-HT neurons selectively without affecting postsynaptic 5-HT_{1A} receptors located on the postsynaptic neurons. This selective destruction produced no significant changes in the drinking behavior of rats in either punished or unpunished sessions of the Vogel conflict test. Furthermore, this destruction altered neither the effect of tansospirone on punished responding in this procedure nor the potency of tansospirone to induce a flat body posture in rats, which is known as the “serotonin behavioral syndrome”. These results suggested that the anticonflict action of tansospirone may be produced, at least in part, by binding to postsynaptic 5-HT_{1A} receptors and activating them as agonists, and not to 5-HT_{1A} autoreceptors located on the cell bodies of 5-HT neurons.

Keywords: Tansospirone, Anxiolytics, 5-HT_{1A} receptors, 5,7-Dihydroxytryptamine, Anticonflict action

The serotonin (5-HT) system has long been implicated in the control of anxiety. Benzodiazepines, predominantly used in the therapy of anxiety, for example, are known to reduce 5-HT turnover and decrease the activity of the central 5-HT neurons (1). The role of 5-HT in anxiety, however, remains to be elucidated, because there is still controversy regarding the role of 5-HT in animal models to predict the anxiolytic activity in man. Recent radioligand binding studies revealed four types of 5-HT receptors in the mammalian brain designated as 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ receptors (2, 3). The 5-HT₁ receptors can be further divided into four distinct subtypes which are presently termed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors (4, 5). Therefore, previous reports studying the role of 5-HT in anxiety should be reviewed again considering the effects on these 5-HT receptor subtypes.

Tansospirone (3 α ,4 β ,7 β ,7 α -hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1*H*-isoindole-1,3 (2*H*) dione dihydrogen citrate) is a non-benzodiazepine compound possessing both potent anxiolytic

properties in animal models (6) and a high potency to bind selectively to 5-HT_{1A} receptors (7, 8). Therefore, this compound may be a useful tool for basic research on the role of 5-HT in anxiety. We have found that spiroperidol (a nonselective 5-HT_{1A} antagonist), but not haloperidol (a D₂-antagonist) or ketanserin (a 5-HT₂ antagonist), inhibits significantly the anticonflict action of tansospirone in Vogel's conflict test (H. Shimizu et al., unpublished data). Therefore, it is suggested that the agonist action of tansospirone on 5-HT_{1A} receptors may produce its anxiolytic activity. It is, however, well-known that there is a functional difference between 5-HT_{1A} receptors in the brain areas receiving a serotonergic input from the raphe nuclei and those in the raphe nuclei (9). Namely, the former are postsynaptic receptors located on the postsynaptic neurons, and the latter are autoreceptors located on the cell bodies and dendrites (but not presynaptic terminals) of 5-HT neurons. A function of the autoreceptors is to trigger feedback mechanisms which exert a negative influence on the neuron impulse flow and the 5-HT

release process in these neurons (10). Tansospirone binds to both postsynaptic receptors and somatodendritic autoreceptors in the same manner (11, 12). Thus, to clarify which receptors mediate the anxiolytic activity of tansospirone, we studied the behavioral effects of tansospirone in 5,7-DHT-treated rats in the present report.

MATERIALS AND METHODS

Male Sprague-Dawley rats (180–200 g) were used in all experiments. These subjects were housed four per cage in a temperature and humidity-controlled room with a 12-hr day-night cycle (illumination from 0800–2000 hours) and received food and water ad libitum except when indicated. Tansospirone dissolved in saline or saline alone was administered in volumes of 5 ml/kg in rats.

5,7-DHT treatment

Rats were pretreated with desipramine (25 mg/kg, i.p.) to protect against the destruction of norepinephrine neurons (13). Fifteen minutes following the pretreatment, the rats were anesthetized with pentobarbital (48 mg/kg, i.p.) and placed in a stereotaxic frame. Fifteen minutes following the pentobarbital administration, 100 μ g of 5,7-dihydroxytryptamine (5,7-DHT), a selective neurotoxin of 5-HT neurons (14), dissolved in 20 μ l of saline, or saline alone for the control rats was injected into the right lateral ventricle. Rats were used, following 15 days and 16 days in their home cages, for behavioral tests (conflict and 5-HT syndrome) and for biochemical confirmation of the lesion (quantitative autoradiography and measurements of monoamines and their metabolite levels), respectively.

Monoamines and their metabolite levels

Rats were sacrificed by decapitation, and then the brains were quickly removed, rinsed in ice-cold saline, and dissected into 5 regions: cerebral cortex, hippocampus, striatum, thalamus and hypothalamus, and mesencephalon. Immediately, the tissues were weighed, frozen in ethanol-dry ice, and stored at -24°C until assayed (within a week). Monoamines and their metabolites were analyzed by the method described in our previous report (15) using high performance liquid chromatography with electrochemical detection (HPLC-ECD). The amount of each compound in a sample was determined by measuring peak heights and comparing them with the known standards. The data from the tissue samples were expressed in nanograms per grams of tissue wet weight.

[^3H]8-OH-DPAT binding

Rats were sacrificed by decapitation, and the brains were quickly removed, rinsed in ice-cold saline, and frozen in ethanol-dry ice. Twenty-micron-thick tissue sections were cut at -15°C using a microtome cryostat, thaw-mounted onto gelatine-coated glass slides, and stored at -25°C until used (for less than 2 weeks). Sections were labeled in vitro according to the procedure of Verge et al. (16). In brief, tissue sections were preincubated at 25°C for 30 min in 0.17 M Tris-HCl buffer (pH 7.4). Slides were then incubated at 25°C for 60 min in the same buffer, containing [^3H]8-hydroxy-2-(di-*n*-propylamino) tetralin ([^3H]8-OH-DPAT, 2 nM), CaCl_2 (4 mM), and clomipramine (0.5 μM), which is a 5-HT reuptake inhibitor. After incubation, washing (2 $\times$ 5 min in ice-cold preincubation buffer and quick dipping in ice-cold distilled water), and drying with cold air, the slides were put in close contact with a sheet of tritium-sensitive film (Sakura, Japan) and allowed to expose in the dark at 4°C for 3 weeks. The nonspecific binding was determined by adding unlabeled 8-OH-DPAT (1 μM) to the incubations. Analysis of the autoradiograms was performed with an image analysis system (Unigraphy UHG-101, Unique Medical, Japan) using a densitometry program. This densitometry program converts optical densities to fmol/mg protein using tritium micro-scales (Amersham, U.K.) co-exposed with the tissue sections. The protein concentration was measured by the method of Lowry et al. (17).

Conflict test

A modification of the method of Vogel et al. (18) was used.

Effect of 5,7-DHT treatment: Both control and 5,7-DHT-treated rats were deprived of water for a 24-hr period prior to the first training session (unpunished session), which always started at between 1000 and 1200 hours. In the session, each animal was placed in a plexiglass conflict test box (38 $\times$ 38 $\times$ 20 cm). A water bottle with a stainless steel spout was fitted onto the outside of one side so that the spout extended 1 cm into the box at a height of 2 cm above the base level. The rat was allowed to explore until it discovered the drinking spout and began to drink, and then the number of licks from the spout within a 3-min period (unpunished responding) was counted. The rats were successively deprived of water until the second session. Twenty-four hours following the unpunished session, which meant 48 hr after the introduction of the water-deprivation, each rat was again placed in the test box. The second session (pre-drug punished session) also lasted for a 3-min period and started automatically when the rat completed 20 licks and received the first mild electric shock

(0.35 mA, 0.5 sec) which was delivered through the spout and the grid floor. Following every 20 unpunished licks, subsequent licking was punished. The number of shocks received within 3 min (punished responding) was counted.

Effect of tansospirone: Only rats displaying suppressed licking (less than 260 licks) due to punishment during the pre-drug punished session in comparison with the unpunished session were included in the study. The rats were successively deprived of water until the next session. Both control rats and 5,7-DHT-treated rats received either tansospirone (0.1, 0.3, and 1.0 mg/kg, s.c.) or saline 2 hr after the pre-drug punished session. One hour after the administration, the 3-min punished session (post-drug punished session) was repeated. The number of shocks received in the post-drug punished session was measured. Each animal was used only once.

5-HT syndrome

Rats were placed singly in a clear plastic cage (50 × 25 × 20 cm). Five minutes later, the rat received tansospirone (0.1, 0.3 and 1.0 mg/kg, s.c.) or saline. Observation sessions of a 15-sec duration began 3 min later and were repeated every 3 min over a period of 30 min. Flat body posture was scored using a ranked intensity scale: 0 = absent, 1 = equivocal, 2 = present and 3 = intense. Each score was summed up over the 10 observation periods.

Drugs

The following drugs were used: Tansospirone citrate,

and 8-OH-DPAT HBr (Sumitomo Pharmaceuticals Co., Ltd.); 5,7-DHT creatinine sulfate, L-NE bitartrate, DA HCl, and HVA (Sigma, U.S.A.); DL-MHPG piperazine, DOPAC, 5-HIAA, 1-octane and sulfonic acid Na (Aldrich, U.S.A.); 5-HT creatinine sulfate (Merck, G.F.R.); DL-isoproterenol HCl (Nacalai Tesque, Japan); and [³H]8-OH-DPAT (183 Ci/mmol, Amersham, U.K.).

Statistical analyses

Data were analyzed by two-way analysis of variance (ANOVA 2 × 2) followed by Tukey's test. Biochemical data were analysed by Student's *t*-test.

RESULTS

Monoamines and their metabolite levels

Table 1 shows the extent of regional depletions of central monoamines and their metabolites, 16 days following the administration of 5,7-DHT. In these subjects, pretreatment with 5,7-DHT significantly decreased the concentrations of 5-HT and its metabolite (5-HIAA) in all brain areas examined, when compared to the saline-treated (control) rats. Particularly in the hippocampus, the levels of 5-HT and 5-HIAA in the 5,7-DHT-treated rats decreased markedly to 6% and 10% of the control rats, respectively. In contrast, the concentrations of NE, DA and its metabolites (DOPAC and HVA) were not significantly altered in any area of the brain. These results show a specific and extensive degeneration of serotonergic terminals in the

Table 1. Effects of the pretreatment with 5,7-DHT on the concentrations of monoamines and their metabolites in various brain regions of rats

Region	Treatment	Concentrations of the monoamines and their metabolites					
		NE	DA	DOPAC	HVA	5-HT	5-HIAA
Cerebral cortex	Control	347 ± 21	455 ± 55	99 ± 13	66 ± 6	618 ± 13	282 ± 9
	5,7-DHT	337 ± 22	449 ± 53	98 ± 12	58 ± 5	233 ± 60**	102 ± 22**
Hippocampus	Control	392 ± 16	37 ± 8	8 ± 3	10 ± 1	437 ± 25	329 ± 23
	5,7-DHT	319 ± 14	22 ± 4	9 ± 1	10 ± 1	26 ± 7**	34 ± 8**
Striatum	Control	216 ± 41	9070 ± 510	1310 ± 66	698 ± 44	446 ± 23	413 ± 12
	5,7-DHT	145 ± 10	8070 ± 186	1270 ± 44	591 ± 36	242 ± 61*	256 ± 52*
Thalamus + Hypothalamus	Control	845 ± 26	247 ± 10	53 ± 4	29 ± 3	539 ± 15	398 ± 12
	5,7-DHT	856 ± 50	225 ± 21	50 ± 5	25 ± 5	267 ± 32**	192 ± 27**
Mesencephalon	Control	584 ± 13	75 ± 4	27 ± 1	20 ± 3	589 ± 10	483 ± 17
	5,7-DHT	558 ± 20	82 ± 20	28 ± 3	17 ± 3	331 ± 53**	240 ± 59**

Data are expressed in ng/g wet tissue; Each value represents the mean ± S.E.M. obtained from five animals. *P < 0.05, **P < 0.01, compared to the corresponding control group (Student's *t*-test).

5,7-DHT-treated rats.

[³H]8-OH-DPAT binding

Figure 1 shows autoradiograms of specific [³H]8-OH-DPAT binding to the hippocampus (A) and the dorsal raphe nucleus (B) in a control rat and a 5,7-DHT treated rat. Table 2 shows specific [³H]8-OH-DPAT binding (fmol/mg protein) in various brain areas calculated from autoradiographic films. In control animals, [³H]8-OH-DPAT binding sites were particularly abundant in the hippocampus, entorhinal cortex, interpeduncular nucleus, lateral septum and dorsal raphe nucleus. No [³H]8-OH-DPAT binding sites could be detected in the caudate putamen, substantia nigra and nucleus accumbens. Also, low to moderate densities of binding sites were found in the other examined structures (Table 2). This regional distribution of [³H]8-OH-DPAT binding

sites was in agreement with those of 5-HT_{1A} receptors in a previous report (17). Pretreatment with 5,7-DHT significantly reduced [³H]8-OH-DPAT binding density in the dorsal raphe nucleus to 63% of the control (Fig. 1B, Table 2). This shows that 5-HT_{1A} autoreceptors were reduced by either a direct neurotoxic effect of 5,7-DHT on serotonergic cell bodies located in the dorsal raphe or a retrograde degeneration of the lesioned 5-HT neurons. In contrast, no significant difference was observed in the binding densities in the hippocampus (Fig. 1A) nor in any area of the brain but the dorsal raphe nucleus (Table 2).

Conflict test

Fourteen days after 5,7-DHT treatment, there was no significant difference in spontaneous drinking behaviors between the control rats and the 5,7-DHT-treated rats.

Fig. 1. Autoradiograms of [³H]8-OH-DPAT binding to the hippocampus (A) and the dorsal raphe nucleus (B) of a control rat and a 5,7-DHT-treated rat. 5,7-DHT was administered by intracerebroventricular injection and animals were sacrificed 16 days later. Coronal sections were taken at (A) = -5.70 mm, (B) = -7.80 mm from the bregma, according to the stereotaxic atlas of Paxinos and Watson (39), and they were then incubated with 2 nM [³H]8-OH-DPAT. Similar results were obtained in five rats in each group. The color scale indicates decreasing binding densities from red to purple.

Table 2. Effects of the pretreatment with 5,7-DHT on 5-HT_{1A} receptors labeled with [³H]8-OH-DPAT in various brain regions

Brain structure	Specific binding (fmol/mg tissue)	
	control	5,7-DHT
Posterior hippocampus		
Dentate gyrus	147 ± 5	141 ± 6
CA field	131 ± 5	135 ± 5
Entorhinal cortex	124 ± 5	119 ± 4
Anterior hippocampus		
Dentate gyrus	115 ± 4	113 ± 4
Interpeduncular nucleus	115 ± 7	114 ± 2
Lateral septum	107 ± 3	114 ± 1
Dorsal raphe nucleus	100 ± 9	62 ± 6**
Amygdala		
Cortical nucleus	71 ± 5	63 ± 4
Central nucleus	35 ± 1	37 ± 2
Ventromedial hypothalamic nucleus	49 ± 2	50 ± 7
Frontal cortex	32 ± 2	39 ± 2
Median raphe nucleus	21 ± 4	16 ± 3
Caudate putamen	n.d.	n.d.
Substantia nigra	n.d.	n.d.
Nucleus accumbens	n.d.	n.d.

Data were calculated from optical densities of the autoradiographic films; Each value represents the mean ± S.E.M. obtained from five animals (3–13 sections). Nonspecific binding was subtracted from all density readings. **P < 0.01, compared to the corresponding control group (Student's *t*-test). n.d.: not detectable.

The number of licks was 26.4 ± 1.0 and 26.6 ± 1.0 (means ± S.E.M.), respectively (Fig. 2). Likewise, in the punished sessions, the number of licks by the 5,7-DHT-treated rats (4.1 ± 0.5) was not significantly different from that of the control rats (5.8 ± 0.8).

Figure 3 shows the effect of tandospirone on the punished responding of the control and 5,7-DHT-treated rats 15 days after saline or 5,7-DHT-treatment. Tandospirone significantly increased the punished responding of the 5,7-DHT-treated rats as well as that of the control rats. The minimum effective dose of tandospirone in this test was 1.0 mg/kg, s.c. in both the control and the 5,7-DHT-treated rats.

5-HT syndrome

Tandospirone induced a flat body posture, one of the components of the "5-HT behavioral syndrome", in a dose-dependent manner in both the control and 5,7-DHT-treated rats. Figure 4 shows the intensities of this behavior induced by various doses of tandospirone in the control and the 5,7-DHT-treated rats. No significant difference in the potency of tandospirone between the two groups was observed.

Fig. 2. The effects of the 5,7-DHT treatment on the drinking behavior of the rats in punished and unpunished sessions of the Vogel conflict test. The number of shocks received in the unpunished session and the punished session was measured 14 days and 15 days following the intracerebroventricular administration of 5,7-DHT, respectively. Each value represents the mean ± S.E.M. obtained from 37 control rats and 48 5,7-DHT-treated rats. *P < 0.01, compared to the corresponding unpunished responding (Tukey's test). (□) control group, (■) 5,7-DHT-treated group.

Fig. 3. The effects of the treatment with 5,7-DHT on the anti-conflict action of tandospirone in the Vogel conflict test. Tandospirone was administered 15 days following the intracerebroventricular injection of 5,7-DHT. The number of shocks received in the punished session was measured 1 hr following the tandospirone administration. Each value represents the mean $\pm$ S.E.M. obtained from eight animals. * $P < 0.01$, compared to the corresponding saline-treated group (Tukey's test). (□) control group, (■) 5,7-DHT-treated group.

DISCUSSION

Both monoamines and their metabolite levels (Table 1) and densities of [^3H]8-OH-DPAT binding (Fig. 1, Table 2) in the brain demonstrated that pretreatment with 5,7-DHT selectively destroyed 5-HT neurons and 5-HT_{1A} autoreceptors located on those cell bodies, without affecting postsynaptic 5-HT_{1A} receptors located on the postsynaptic neurons. This selective destruction of 5-HT neurons with 5,7-DHT had no significant effect on the spontaneous drinking behaviors of the rats (Fig. 2). Also, the drinking behaviors of these 5,7-DHT-treated rats, like those of the control rats, are markedly suppressed by electric shock (Fig. 2). These results were in agreement with the data by Thiebot et al. (19) and Commissaris et al. (20), who reported no increase in the punished behavior of 5,7-DHT-treated rats in the Geller-Seifter conflict test and the Vogel conflict test, respectively. However, Tye et al. (21) have reported that the administration of 5,7-DHT into the ventromedial tegmentum produced anticonflict action in food-deprived rats, and that the anxiolytic effects of benzodiazepines may result from a reduction in the activity of the serotonergic neuron system. On the other hand, in the studies of escape behavior after aversive electrical stimulation of the dorsal periaqueductal grey region of

Fig. 4. The effects of the treatment with 5,7-DHT on flat body posture induced by tandospirone in rats. Tandospirone was administered 15 days following the intracerebroventricular injection of 5,7-DHT. Following the tandospirone administration, the intensities of a flat body posture were measured during a 30-min period. Each value represents the mean $\pm$ S.E.M. obtained from eight animals. (□) control group, (■) 5,7-DHT-treated group.

the brain, it has been reported that a reduction of 5-HT systems enhances the aversive response (22), and that 5-HT agonists containing 5-HT itself, in contrast, show antiaversive action (23). This discrepancy may be due to differences in the specificity or manner of approach that has been used either to reduce or to enhance serotonergic mechanisms, or differences in the methods used to estimate the anxiolytic activity. The results obtained in the present study suggest at least that a reduction in the activity of 5-HT neuron systems does not produce anxiolytic action in rats.

Tricklebank et al. (24) have reported that a flat body posture in rats is mediated by postsynaptic 5-HT_{1A} receptors. Tandospirone induced this behavior in 5,7-DHT-treated rats as well as in control rats. Since 5,7-DHT treatment had no significant effect on postsynaptic 5-HT_{1A} receptors (Table 2), this is in agreement with the result reported by Tricklebank et al. Likewise, the treatment with 5,7-DHT failed to alter the anticonflict activity of tandospirone in rats (Fig. 3). This suggests that the anticonflict action of tandospirone is not due to its effect on 5-HT_{1A} autoreceptors located on 5-HT neurons. Since we have presented the following evidence that tandospirone shows anticonflict action, at least in part, by binding to central 5-HT_{1A} receptors and activating them as agonists, these findings suggest that the anticonflict action of tandospirone may be mediated by postsynaptic 5-HT_{1A} receptors. That is: a) tandospirone binds selectively to central 5-HT_{1A} recep-

tors with high affinity (7, 8, 11, 12), b) tandospirone induces the "serotonin behavioral syndrome" (Fig. 4 of this paper and ref. 25), c) tandospirone reduces 5-HT turnover rate in the rat brain (15), d) tandospirone inhibits forskolin-stimulated adenylate cyclase activity in rat hippocampal membranes, and this effect is antagonized by a 5-HT_{1A} antagonist (26), e) the inhibition of hippocampal rhythmical slow activity by tandospirone is antagonized by a 5-HT_{1A} antagonist (27), and f) the anticonflict action of tandospirone is inhibited by a 5-HT_{1A} antagonist (H. Shimizu et al., unpublished data).

This conclusion is also supported by the results reported by Kataoka et al. (28), who showed that the administration of tandospirone into the dorsal hippocampus produced a potent anticonflict action in rats, and Godbout et al. (29), who demonstrated that the sensitivity of the somatodendritic 5-HT_{1A} receptors was reduced by the sustained administration of tandospirone and that an augmented tonic activation of postsynaptic 5-HT_{1A} receptors would produce the anxiolytic effect. In regard to other 5-HT_{1A} receptor-related anxiolytics, it has been reported that buspirone and ipsapirone produced anxiolytic activity in raphe-lesioned animals (30) and in animals whose 5-HT neurons are destroyed by *p*-chloroamphetamine (31). Moreover, Kostowski et al. (32) have reported that the administration of buspirone into the hippocampus produced anxiolytic action in an elevated plus-maze test. On the other hand, there are also some reports suggesting the involvement of presynaptic 5-HT_{1A} receptors in the anxiolytic activity of these 5-HT_{1A} receptor-related anxiolytics. Namely, it has been reported that the anxiolytic effect of buspirone or gepirone was antagonized in 5,7-DHT-treated animals in a conflict test (33) and a two-compartment exploratory test (34). It also has been reported that the administration of buspirone and ipsapirone into the raphe nuclei produced anxiolytic actions in several animal models of anxiety (34, 35). The pharmacological profiles of buspirone and ipsapirone are not the same as that of tandospirone. Buspirone and ipsapirone, for example, show much higher D₂- and α_1 -antagonist activities, respectively, than tandospirone (36). Moreover, intrinsic activities of buspirone and ipsapirone for 5-HT_{1A} receptors are lower than that of tandospirone (H. Shimizu et al., unpublished data). Therefore, such different pharmacological properties of the 5-HT_{1A} receptor-related anxiolytics might lead to the above-mentioned conflicting results. It is, however, difficult to compare these results because of the different experimental conditions used in these studies, so that the cause of these contradictory findings is not clear. Therefore, further studies are necessary to clarify which 5-HT_{1A} receptors mediate anxiolytic activity of the 5-

HT_{1A} receptor-related anxiolytics. However, as far as tandospirone is concerned, the present results suggest the importance of postsynaptic 5-HT_{1A} receptors in its anxiolytic mechanism.

The brain region containing the highest density of postsynaptic 5-HT_{1A} receptors has been shown to be the hippocampus (Table 2 of this paper and ref. 9), which has long been thought to be implicated in the control of anxiety. Gray (37) has reported that activated neuronal activity is observed in the hippocampus of anxious animals and that anxiolytics share the property of having inhibitory effects in the hippocampus. Our previous studies have shown that tandospirone inhibits neuronal activities in the rat hippocampus through postsynaptic 5-HT_{1A} receptors (27, 38). Moreover, as previously mentioned, the microinjection of tandospirone into the dorsal hippocampus produced the anticonflict action in rats (28). In the light of these findings, it is suggested that the agonist action of tandospirone on postsynaptic 5-HT_{1A} receptors, especially in the hippocampus, may produce its anxiolytic activity.

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