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5-HT_{2A} Serotonin Receptors in the Brain of Rats and Mice Hereditarily Predisposed to Catalepsy

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KULIKOV, A. V., D. F. AVGUSTINOVICH, V. G. KOLPAKOV, G. B. MASLOVA AND N. K. POPOVA. *5-HT_{2A} serotonin receptors in the brain of rats and mice hereditarily predisposed to catalepsy.* PHARMACOL BIOCHEM BEHAV **50**(3) 383-387, 1995.—Specific binding of [^{3}H]ketanserin to 5-HT_{2A} serotonin receptor sites in the corpus striatum and frontal cortex and the effect of 5-HT_{2A} antagonists in rats and mice hereditarily predisposed to catalepsy has been studied. Cyproheptadine inhibited the expression of inherited catalepsy in rats and mice, whereas more selective 5-HT_{2A} antagonists, ritanserin and ketanserin, failed to affect the catalepsy. A decrease in [^{3}H]ketanserin-specific binding site density in the striatum of cataleptic animals compared to their noncataleptic counterparts was found. It was suggested that the decrease in the density of 5-HT_{2A} receptor binding sites in the striatum represents a result of their downregulation due to increased serotonergic neurotransmission in the striatum of cataleptic animals.

Hereditary catalepsy 5-HT_{2A} receptor binding sites Striatum Cyproheptadine Ritanserin Ketanserin

SEROTONIN 5-HT_2 binding sites, recently renamed as 5-HT_{2A} serotonin receptors (11), are designated as those labelled by radioactive serotonin antagonists: [^{3}H]spiperone, [^{3}H]LSD-25, and [^{3}H]ketanserin (19). They show high binding affinity for various serotonin antagonists and low binding affinity for serotonin agonists (26). The highest level of 5-HT_{2A} binding sites was found in cerebral cortex and caudate (25). 5-HT_{2A} receptors are coupled to cellular phosphatidylinositol turnover (4). Serotonin produces a slow depolarization of cortical neurons via these receptors (6). Several components of the "serotonin syndrome," including behavioral excitation, tryptamine-induced clonic seizures, bilateral forepaw treading, and head twitches, were shown to be mediated by 5-HT_{2A} receptor sites (21,28). Considerable progress in the molecular genetics of 5-HT_{2A} serotonin receptor has been made in recent years. The intronless gene encoding the rat 5-HT_{2A} receptor was cloned and was shown to be a member of the superfamily of G-binding receptors (30). Recently, the 5-HT_{2A} receptor encoding gene was mapped on human chromosome 13 and on mouse chromosome 14 (10,32). However, the role of 5-HT_{2A} receptors in genetically defined serotonin-dependent behaviors are still unknown.

Hereditary catalepsy in rats and mice is a good model for studying the association of the receptors with behavior. Catalepsy is an active immobility response to certain stimuli, and is defined as a failure to correct an externally imposed posture. It is an element of catatonic syndrome of Parkinson's disease, schizophrenia (7,8), and a side effect of antipsychotic drug treatment (9). A rat strain was successfully bred for predisposition to catalepsy (14), and significant interstrain differences in susceptibility to restraint-induced catalepsy in mice was found (16). On the other hand, hereditary catalepsy in both rats and in mice seems to be regulated by the brain serotonergic mechanism. A significant increase in activity of the rate-limiting enzyme of serotonin biosynthesis, tryptophan hydroxylase, in the striatum of rats, selectively bred for predisposition to catalepsy, has been found (15,17). Highly susceptible to restraint-induced catalepsy, mice of the CBA strain also had an increased tryptophan hydroxylase activity in the striatum compared to mice of other noncataleptic strains (18).

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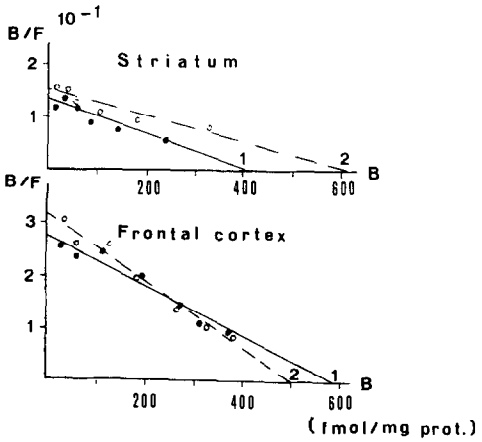

FIG. 1. Scatchard plots for specific [^{3}H]ketanserin binding in the neostriatum and frontal cortex of genetically predisposed to catalepsy rats (solid line, black circles) and noncataleptic Wistar rats (dashed line, open circles).

Earlier we found decreased intensity of 5-hydroxytryptamine-induced head twitches in rats hereditarily predisposed to catalepsy and suggested an alteration of 5-HT_{2A} serotonin receptors in the brain of the cataleptic rats (27).

The following experiments were conducted to compare the binding characteristics of 5-HT_{2A} serotonin receptors in the brain of cataleptic and noncataleptic rats and mice and evaluate the effect of 5-HT_{2A} antagonists on the expression of inherited catalepsy.

METHOD

Animals

Males (3 months of age, weighing 250 g) of the rat strain selectively bred for 25 generations for predisposition to catalepsy from a random bred Wistar stock (14) were used. As controls, noncataleptic Wistar rats of respective age and weight kept under the same conditions were used. Catalepsy was estimated by the time during which an animal being lifted with a stick by its forelegs maintained this imposed vertical position. Every animal was subjected to five tests with 5-min intervals between the tests. Rats that maintained the posture for no less than at least 10 s in three successive tests were considered cataleptics.

Males (2 months of age, weighing 20 g) of the cataleptic CBA/Icg mouse strain and noncataleptic C57BL/6Icg strain (16) maintained by close inbreeding for 20 years at the Institute of Cytology & Genetics (Novosibirsk) were used. Restraint-induced catalepsy was induced as originally described by Amir et al. (1). Animals were firmly pinched by fingers for 5 s at the scruff of the neck. They were then placed on parallel bars, with the front paws situated at a 45° angle above the hind paws, and were released gently. The duration of freezing was recorded. Every animal was subjected to 10 tests. Each test was limited to 2 min. The intervals between the tests were 2 min. A mouse was considered as cataleptic if the time of immobility was above at least 20 s in three successive tests. At this test about 56% of CBA males were defined as cataleptic, whereas none of C57BL males demonstrated freezing (16).

A day before the experiments rats and mice were isolated in individual cages to assess the baseline predisposition to catalepsy and the density of 5-HT_{2A} receptors in the brain.

Radioligand Binding

Intact rats and mice were decapitated; their brains were removed and chilled rapidly on ice. Frontal cortex and striatum were isolated, rapidly frozen, and stored under liquid nitrogen until use.

Specific binding of [^{3}H]ketanserin to membranes from striatum and cortex was assayed as described earlier (29). Brain tissues were homogenized in 40 vol. of cold 50 mM Tris HCl, pH 7.6. The homogenates were put into snow for 15 min to complete cell lysis and then spun at 20,000 × *g* for 30 min (+4°C). The pellets were resuspended in the same volume of the buffer and spun at 20,000 × *g* for 30 min (+4°C). The final pellets were resuspended in 20 vol. of the buffer. Binding assay consisted of 0.9 ml of the suspension, 0.05 ml of 0.125–4 nM [^{3}H]ketanserin (64.1 Ci/mmol, Du Pont, Germany), and 0.05 ml of the buffer or "cold" ritanserin (10^{-6} M, Janssen Parmaceutica, Belgium) as the displacing drug. Following incubation at 37°C for 15 min, samples were rapidly filtered through Whatman glass fiber GF/B filters with washing 3 × 5 ml of cold buffer. The filters were then placed in glass vials containing 4 ml of a dioxane scintillator and their radioactivity was measured by a Delta-300 scintillation counter at 40% efficiency. Specific binding was defined as the difference between [^{3}H]ketanserin binding in the absence (total binding) and in presence of 10^{-6} M cold ritanserin (nonspecific binding) and expressed as fmol/mg of protein.

Drugs

The 5-HT_{2A} antagonists ritanserin (Janssen Pharmaceutica, Belgium; 1.0 and 2.0 mg/kg), ketanserin (Janssen Pharmaceutica, 2.0 mg/kg), and cyproheptadine (Serva, Germany, 10 mg/kg) were used. The doses of ketanserin and cyproheptadine were selected according to Neal-Beliveau et al. (24) and Maj et al. (23). Ritanserin and ketanserin were dissolved in 1% tartaric acid and cyproheptadine was dissolved into water. All drugs and vehicles were given IP and their effect on catalepsy was estimated 1 h after the drug administration. Each animal was treated with respective vehicle (1% tartaric acid solution or water) and on the next day with a drug. Vehicle-treated animals were used as controls. Each drug was injected

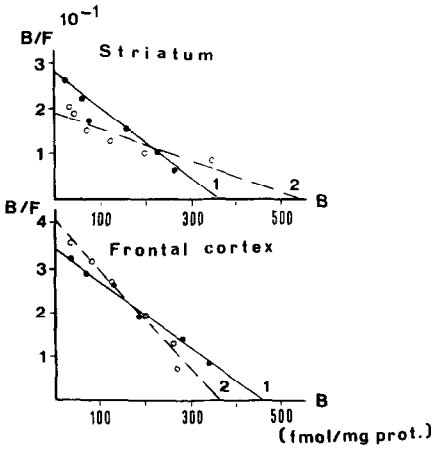

FIG. 2. Scatchard plots for specific [^{3}H]ketanserin binding in the neostriatum and frontal cortex of cataleptic CBA mice (solid line, black circles) and noncataleptic C57BL mice (dashed line, open circles).

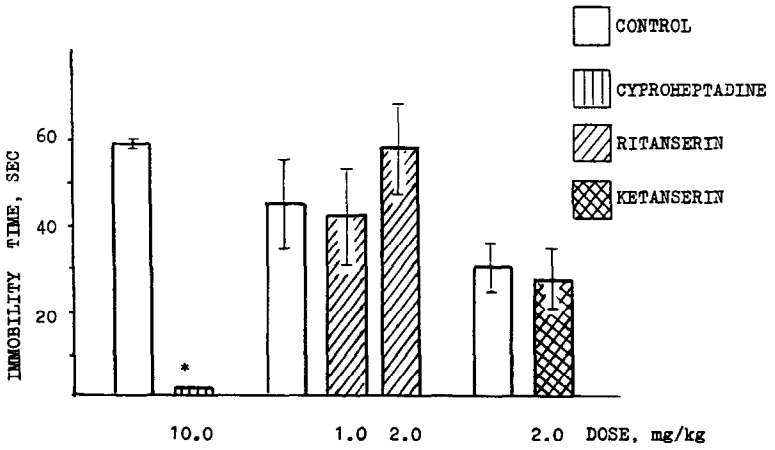

FIG. 3. Effect of cyproheptadine, ritanserin, and ketanserin on the immobility time in rats predisposed to catalepsy. Catalepsy was tested 1 h after cyproheptadine (10 mg/kg), ritanserin (1, 2 mg/kg), or ketanserin (2 mg/kg) administration. *$p <$ 0.01 vs. respective vehicle-treated animals.

via a separate syringe and needle. Different animals were used to test the effect of different drugs.

Statistics

The B_{max} and K_d values for [^{3}H]ketanserin-specific binding and their MSEs were calculated by the least square method (5). Drug effect on catalepsy was tested by ANOVA and Student's *t*-test for dependent variables using the CSS/PC set of statistic programs.

RESULTS

The Comparison of [^{3}H]Ketanserin-Specific Binding in the Brain of Cataleptic and Noncataleptic Animals

The B_{max} value for [^{3}H]ketanserin-specific binding in the striatum of rats bred for predisposition to catalepsy was significantly lower than that in noncataleptic Wistar rats [408.3 ± 49.5 fmol/mg in cataleptics and 612.9 ± 76.1 fmol/mg in Wistar, $t(52) = 2.25, p < 0.05$]. No difference in the apparent K_d as well as in B_{max} for the specific [^{3}H]ketanserin binding to membranes from frontal cortex between cataleptic and Wistar rats was observed [589.5 ± 40.34 fmol/mg in cataleptics and 502.8 ± 25.69 fmol/mg in Wistar, $t(50) = 1.81$, $p > 0.05$] (Fig. 1). Lower B_{max} for the ligand binding was shown in the striatum of cataleptic CBA mice compared with noncataleptic C57BL mice. B_{max} for [^{3}H]ketanserin binding in the striatum of CBA mice was 370.9 ± 19.5 fmol/mg, whereas in the same brain structure of C57BL mice it was 545.6 ± 67.7 fmol/mg, $t(52) = 2.48, p < 0.05$. At the same time, an even higher B_{max} value for specific [^{3}H]ketanserin binding in cortex of CBA mice was found [461.78 ± 20.59 fmol/mg in CBA and 361.31 ± 14.84 fmol/mg in C57BL, $t(52) = 4.0, p < 0.01$] (Fig. 2).

The Effect of 5-HT$_{2A}$ Antagonists on the Duration of Catalepsy

Cyproheptadine (10 mg/kg, IP), 1 h after its IP administration to 10 cataleptic rats, produced complete inhibition of freezing ($t = 48.1, p < 0.01$) (Fig. 3). A selective 5-HT$_{2A}$ antagonist, ritanserin, did not affect immobility time in rats predisposed to catalepsy at all doses tested, $F(2, 10) = 1.7, p > 0.05$ (Fig. 3). Another selective antagonist of this receptor type, ketanserin, did not influence the immobility time either ($t = 0.24, p > 0.05$) (Fig. 3).

Cyproheptadine induced a twofold decrease of immobility time 30 min after its IP administration to 15 cataleptic CBA mice ($t = 7.3, p < 0.01$) (Fig. 4). At the same time, ritanserin (2 mg/kg) failed to affect freezing time in cataleptic CBA mice ($t = 1.6, p > 0.05$) (Fig 4).

DISCUSSION

It was shown that a 5-HT$_{2A}$ antagonist, cyproheptadine, produced a significant decrease of immobility time in both rats and mice hereditarily predisposed to catalepsy. However, the more selective 5-HT$_{2A}$ antagonist, ritanserin, did not affect the expression of the catalepsy in rats and mice. It should be noted that similar results were observed on neuroleptic-induced catalepsy. It was shown that ritanserin failed to affect catalepsy induced by dopamine receptor antagonists raclopride and SCH 23390 (33), and haloperidol (Kulikov and Maslova, unpublished data). Ketanserin did not affect catalepsy elicited by the D_2 dopamine receptor antagonist, YM0 9151-2

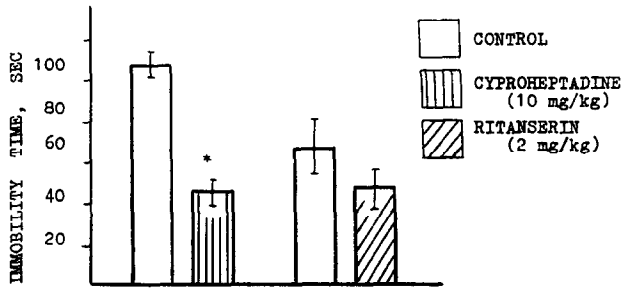

FIG. 4. Effect of cyproheptadine and ritanserin on the immobility time in cataleptic CBA mice. Catalepsy was tested 1 h after cyproheptadine (10 mg/kg) or ritanserin (2 mg/kg) administration. *$p <$ 0.01 vs. respective vehicle-treated animals.

(2). However, less selective 5-HT_{2A} antagonists, cyproheptadine (23), methysergide (3), and mesulergine (9), reversed neuroleptic-induced catalepsy. Collectively, these data strongly suggest that blockade of 5-HT_{2A} serotonin receptors does not influence the expression of inherited as well as neuroleptic-induced catalepsy. The inhibitory effect of cyproheptadine on hereditary and neuroleptic-induced catalepsy seems to be due to its action on some nonserotonergic mechanisms, probably histaminergic, because cyproheptadine, in contrast to ritanserin and ketanserin, has a high affinity to H_1 histamine receptors (19), which were shown to be involved in the regulation of catalepsy (12).

At the same time, a lower density of the sites of [^{3}H]ketanserin specific binding in the striatum of cataleptic rats and mice compared with their noncataleptic counterparts was found. Although [^{3}H]ketanserin was developed as the most selective radioligand to label 5-HT_{2A} receptors (19), about 40% of [^{3}H]ketanserin-specific binding in the striatum can be attributed to sites other than 5-HT_{2A} receptors, with K_d = 14.7 nM (20). However, the relatively low K_d values for [^{3}H]ketanserin-specific binding (about 3 nM in rats and 2 nM in mice) observed in our experiments suggest that at these conditions [^{3}H]ketanserin in the striatum mainly binds to 5-HT_{2A} receptor sites. Therefore, the decrease in [^{3}H]ketanserin-specific binding in the striatum of cataleptic animals does reflect a decrease in 5-HT_{2A} receptor density in this brain structure of cataleptics.

The decrease in 5-HT_{2A} receptor sites in cataleptics was shown in the striatum, whereas in the frontal cortex of the cataleptic animals an increased density of 5-HT_{2A} sites was found. The striatum has been implicated as the major brain structure involved in antipsychotic-induced catalepsy (13). In animals with hereditary predisposition to catalepsy, an increased tryptophan hydroxylase activity was demonstrated selectively in the striatum (15,17,18), suggesting that inherited catalepsy resulted from increased serotonergic transmission in the striatum (17). Drugs increasing synaptic serotonin were shown to cause downregulation of 5-HT_{2A} serotonin receptors in the brain (31). Therefore, the decrease in 5-HT_{2A} receptor density may be considered as a result of their downregulation due to an activation of serotonergic neurotransmission in the striatum. The lack of an anticataleptic effect of selective 5-HT_{2A} receptor antagonists evidently indicates that the catalepsy is not associated with the activation of this type of serotonin receptor. However, the possibility that 5-HT_{2A} receptors, when activated, serve to prevent catalepsy cannot be ruled out. Recently, it has been shown that 5-HT_{2A} agonists DOI (9) and DOB (24) reversed haloperidol-induced catalepsy in rats. This suggests that the decreased 5-HT_{2A} receptor density found in the neostriatum of the cataleptic-prone rats and mice plays a role in the expression of hereditary catalepsy.

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