EXPERIMENTAL GENETICS

Contribution of 5-HT_{1A} Serotonin Receptors of the Brain to the Regulation of Hereditary Catalepsy

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Selective agonists 5-HT_{1A} of serotonin receptors (8-OH-DPAT and flezinoxan) had an inhibitory effect on the manifestation of hereditary catalepsy in mice and rats. No differences were revealed in specific binding of ^{3}H-8-OH-DPAT to 5-HT_{1A} receptors in the striatum of either cataleptic or noncataleptic mice and rats. Nonetheless, an increase of the density of these receptors was observed in the frontal cortex of CBA mice predisposed to catalepsy in comparison with mice of the noncataleptic C57Bl strain. The data indicate a contribution of 5-HT_{1A} receptors to the regulation of hereditary catalepsy.

Key Words: *5-HT_{1A} receptors; hereditary catalepsy; flezinoxan*

Serotonin 1A receptors (5-HT_{1A}), characterized by a high affinity to serotonin agonists and labeled with ^{3}H-8-OH-DPAT [14], contribute to transmission autoregulation in the serotoninergic synapse [7]. Serotonin receptor agonists 5-HT_{1A} lower the pulsed activity of serotoninergic neurons in the nuclei of the midbrain suture and suppress the depolarization-evoked release of transmitter in the synaptic cleft [6]. The influence of serotonin receptor 5-HT_{1A} agonists on the manifestation of haloperidol-induced catalepsy has been demonstrated [5,13].

The present research aimed to elucidate the effects of 5-HT_{1A} receptor agonists on the severity of hereditary catalepsy and to perform a comparative analysis of 5-HT_{1A} receptor binding in the brain of animals with genetically determined differences in terms of catalepsy manifestations.

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MATERIALS AND METHODS

Experiments were carried out with adult male rats aged 2 months and weighing 250 g (35 generations of breeding) selected for predisposition to catalepsy at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences [10]. Catalepsy was induced by raising the front limbs of the animal with a stick and assessed by the time the rat maintained the preset vertical posture. Wistar males matched for age and body weight were controls. In one series of experiments adult male mice of the cataleptic CBA/Icg strain aged 2 months and weighing 20 g [2] were used. Catalepsy was induced by pinching the skin of the nape of the neck for 5 seconds and placing the animal on parallel bars. Catalepsy was assessed by the time of maintenance of the preset posture [2]. C57Bl/6J mice, which never "froze" to a standstill under such conditions served as controls [2]. Two days before the experiment the animals were isolated in individual cages to rule out the group ef-

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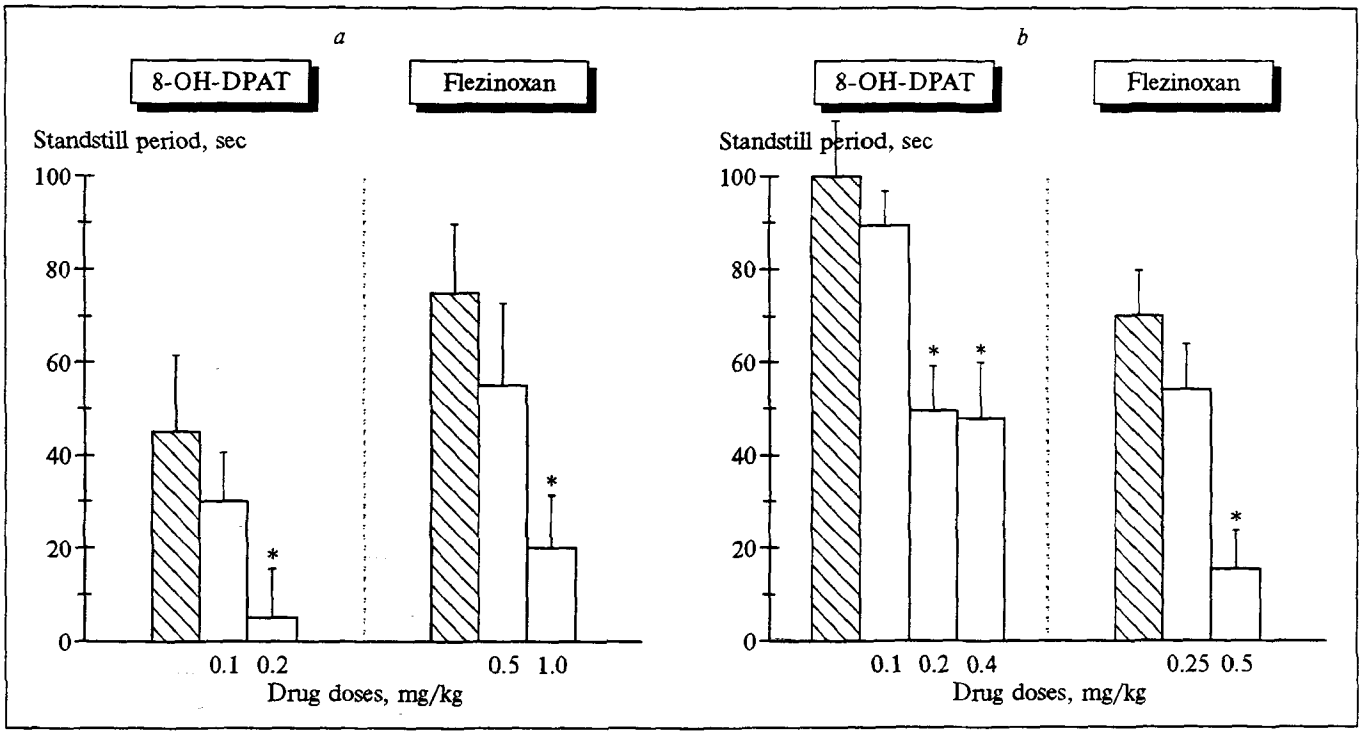

Fig. 1. Effects of different doses of 5-HT_{1A} selective agonists of serotonin receptors 8-OH-DPAT and flezinoxan on the duration of the standstill reaction in cataleptic rats (*a*) and CBA mice (*b*). Number of measurements: 7. Asterisk shows reliable differences ($p<0.05$) from the control.

fect. For characterization of 5-HT_{1A} serotonin receptors the animals were decapitated, the brain was rapidly removed in the cold, and the striatum, a structure associated with the genesis and development of catalepsy [8,9], and the anterior cortex were isolated. The material was frozen in liquid nitrogen and stored at -70°C for a week. The characteristics of 5-HT_{1A} receptors in the brain were assessed by the specific binding of ^{3}H-8-OH-DPAT (240 Ci/mmol, Amersham) using the standard radioligand method [14]. Seven concentrations of the isotope were used, from 0.06 to 4.0 nM. In pharmacological experiments two highly selective 5-HT_{1A} agonists were used: 8-OH-DPAT (0.1, 0.2, and 0.4 mg/kg, Research Biochem., Inc.) and flezinoxan (0.25, 0.5, and 1.0 mg/kg, Duphar). The drugs were dissolved in normal saline and injected intraperitoneally. The effect was observed 1 h after injection. Normal saline was injected to control animals. Drug effects during the rigidity period were tested using ANOVA and Student's test for paired observations. The B_{max} and K_d receptor binding values and errors in these values were estimated by the method of least squares [1] and tested by Student's t test.

TABLE 1. Specific Binding of ^{3}H-8-OH-DPAT in the Striatum and Frontal Cortex of Rats and Mice with Genetically Determined Differences in Predisposition to Catalepsy ($M \pm m$)

Brain area	Genotype	Number of experiments	B_{max}, fmol/mg	K_d, nmol
		Rats		
Striatum	Cataleptics	18	28.4±2.8	1.0±0.2
	Control	18	32.1±2.4	1.1±0.1
Cortex	Cataleptics	21	88.6±4.1	0.8±0.1
	Control	21	86.8±3.8	0.8±0.1
		Mice		
Striatum	C57Bl	18	39.3±2.2	1.4±0.1
	CBA	18	40.0±1.6	1.5±0.1
Cortex	C57Bl	21	82.1±4.6	1.7±0.1
	CBA	21	107.1±4.2*	0.9±0.1*

Note. Asterisk shows $p<0.001$ between CBA and C57Bl.

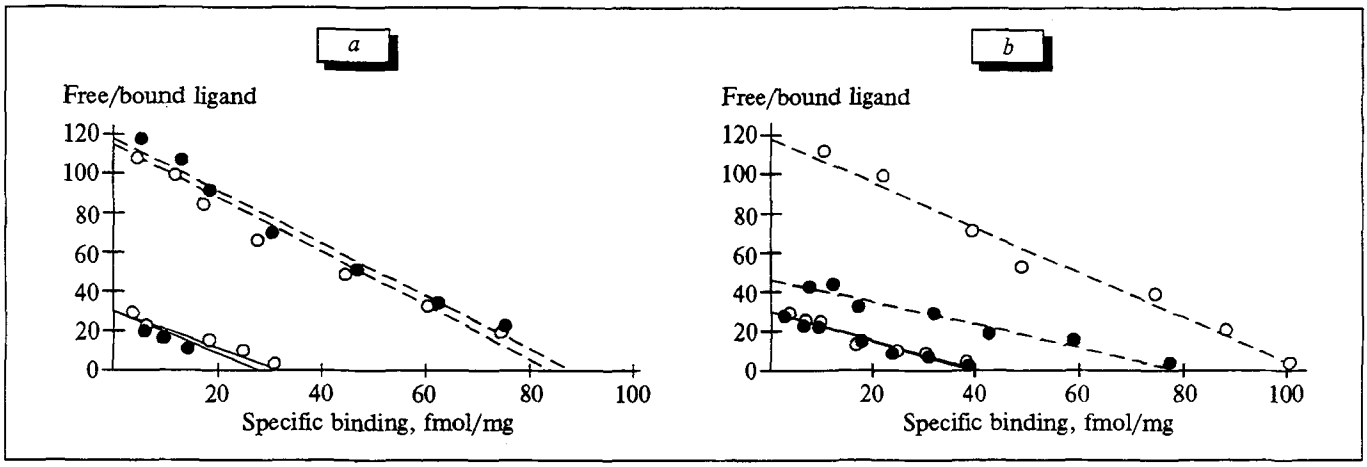

Fig. 2. Scatchard diagrams representing $^3H-OH-DPAT$ specific binding to $5-HT_{1A}$ serotonin receptors in the striatum and frontal cortex of the brain of rats (*a*) and mice (*b*) with hereditery differences in predisposition to catalepsy. Solid lines: striatum, broken lines: frontal cortex. Black circles: control: *a*) Wistar rats, *b*) C57Bl mice. White circles on *a*: rats hereditarily predisposed to catalepsy; on *b*: cataleptic CBA mice. Every point represents the mean value estimated from the results of 3 measurements.

RESULTS

8-OH-DPAT caused an 8-fold reduction of the period of cataleptic rigidity in rats selected for predisposition to catalepsy (Fig. 1, *a*, $p<0.01$) 1 h after the drug was injected in a dose of 0.2 mg/kg. The other $5-HT_{1A}$ agonist, flezinoxan, also shortened the period of rigidity after injection in a dose of 1.0 mg/kg (Fig. 1, *a*, $p<0.05$). These drugs had a similar inhibitory effect on the manifestation of pinch-induced catalepsy in CBA mice (Fig. *1*, *b*). 8-OH-DPAT clearly reduced the "standstill" time ($F(3.15)=7.5$, $p<0.01$), a reduction of catalepsy being observed after the drug was injected in doses of 0.2 and 0.4 mg/kg.

Flezinoxan reliably inhibited the standstill time 1 h after its injection in a dose of 0.5 mg/kg. Hence, $5-HT_{1A}$ receptors are involved in regulating the manifestation of hereditary catalepsy in rats and mice: their activation leads to a suppression of the standstill reaction.

No reliable difference was observed between cataleptic rats and Wistar rats in the B_{max} ($p>0.05$) and K_d ($p>0.05$) values of radioligand $5-HT_{1A}$ specific binding to striatum receptors, nor did the kinetic characteristics of $5-HT_{1A}$ receptor binding in the cortex of Wistar and cataleptic rats differ (Fig. 2, *a*, Table 1). Specific binding of ^3H-8-OH-DPAT in the striatum of cataleptic CBA mice did not differ from that in noncataleptic C57Bl mice either in B_{max} ($p>0.05$), or in K_d ($p>0.05$). At the same time, the B_{max} value in the frontal cortex of CBA mice was reliably higher ($p<0.001$) and the K_d value reliably lower ($p<0.001$, Fig. 2, *b*, Table 1) than in noncataleptic C57Bl mice.

The inhibitory effect of selective $5-HT_{1A}$ agonists on the manifestation of hereditary catalepsy in both rats and mice revealed in our experiments indicates that receptors of this type help regulate this form of catalepsy. Previously $5-HT_{1A}$ agonists were shown to be capable of alleviating catalepsy induced by the neuroleptic haloperidol [5,13]. The similarity of the effects of $5-HT_{1A}$ agonists on the manifestation of hereditary and neuroleptic-induced catalepsy indicate the involvement of $5-HT_{1A}$ serotonin receptors in the basal mechanism of catalepsy. The absence of differences in the number and affinity of $5-HT_{1A}$ serotonin receptors in the striatum of animals with genetically determined differences in predisposition to catalepsy confirms that a genetic predisposition to catalepsy is not associated with hereditary changes in the expression or affinity of receptors of this type in the striatum. This result is somewhat unexpected, because it is in the striatum of cataleptic animals that increased activity of tryptophan hydroxylase, the key enzyme in serotonin biosynthesis, has been detected [3,4,11,12,15]. At the same time, we cannot rule out the possibility of an altered expression of $5-HT_{1A}$ receptors in other brain structures that are also involved in the regulation of catalepsy. The increased density of $5-HT_{1A}$ serotonin receptors which we found in the frontal cortex of the brain of mice with a hereditary predisposition to catalepsy confirms such a hypothesis.

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