GENETICS

Effect of Selective Agonist of Serotonin 5-HT$_{1A}$ Receptors on Defensive Behavior in Mice with Different Predisposition to Catalepsy

D. V. Bazovkina, E. E. Terenina*, and A. V. Kulikov

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We studied the effect of activation of serotonin 5-HT$_{1A}$ receptors with selective agonist 8-OH-DPAT (0.1, 0.5, and 1.0 mg/kg) on intraspecies aggression and freezing reaction (catalepsy) in male mice of catalepsy-resistant AKR/J and two catalepsy-prone strains CBA/Lac and congenic AKR.CBA-D13Mit76. The latter strain differs from AKR strain only by terminal chromosome 13 fragment transferred from CBA strain and containing a locus determining predisposition to catalepsy and a gene encoding 5-HT$_{1A}$ receptor. 8-OH-DPAT in a low dose (0.1 mg/kg) affecting primarily presynaptic receptors suppressed aggressive behavior in CBA mice, but had no effect on the time of cataleptic freezing. At the same time, this dose of the drug produced no significant effect on aggression in AKR and AKR.CBA-D13Mit76 mice, but significantly attenuated freezing in AKR.CBA-D13Mit76 mice. High doses of 8-OH-DPAT (0.5 and 1 mg/kg) which affected mainly postsynaptic receptors inhibited catalepsy in CBA and AKR.CBA-D13Mit76 mice and in a dose of 1 mg/kg it suppressed aggression in all tested mouse strains. We concluded that the genome of the recipient strain (AKR) modulated the involvement of 5-HT$_{1A}$ receptors into the regulation of aggression and catalepsy in mice.

Key Words: *5-HT$_{1A}$ receptor; 8-OH-DPAT; congenic mice; aggression; catalepsy*

Defensive behavior plays an important role in survival of an animal and a species on the whole. It appears when the animal confronts a real or presumptive danger. Two basic defensive strategies are distinguished: aggression (active defense) and freezing or catalepsy (passive defense) [4]. The latter is a state of long-term immobility accompanied by plastic muscular tonus [6].

The inhibiting effect of 5-HT$_{1A}$ serotonin receptor agonists on catalepsy and aggression was experimentally proven [4,8,9]. 5-HT$_{1A}$ receptors can have both postsynaptic (frontal cortex and hippocampus) and presynaptic localization (raphe nuclei, somatodendritic 5-HT$_{1A}$ autoreceptors) [5]. Low doses of selective 5-HT$_{1A}$ receptor agonists affect primarily presynaptic receptors (due to their higher sensitivity), while high doses activate mainly postsynaptic receptors [5,10]. The antiaggressive effect of agonists can be mediated through both pre- and postsynaptic 5-HT$_{1A}$ receptors [9], whereas their anticataleptic effects are related primarily to presynaptic receptors [11].

Recently, a 59-70 cM fragment of mouse chromosome 13 containing a locus determining predisposition to catalepsy and a gene encoding 5-HT$_{1A}$ receptors was

Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia; *Laboratory of Psychoneuroimmunology, Nutrition, and Genetics (PsyNuGen) UMR 1286 INRA, Bordeaux, France. ***Address for correspondence:*** drinterf@ngs.ru. D. V. Bazovkina

transferred from cataleptic CBA/Lac mouse strain into the genome of catalepsy-resistant AKR/J mouse strain. Pronounced catalepsy similar to that observed in CBA mice was observed in 50% mice of congenic AKR. CBA-D13Mit76 strain.

Here we compared the effects of low and high doses of selective 5-HT_{1A} receptor agonist 8-OH-DPAT on intermale aggression in CBA, AKR, and AKR. CBA-D13Mit76 mice and cataleptic freezing in CBA and AKR.CBA.D13Mit76 mice.

MATERIALS AND METHODS

Experiments were performed on mature male CBA, AKR/J, and AKR.CBA-D13Mit76 mice. CBA and AKR mouse strains are maintained at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences for more than 40 years by close inbreeding. AKR.CBA-D13Mit76 mouse strain was obtained by transfer of chromosome 13 fragment labeled by D13Mit76 microsatellite (61 cM) linked with catalepsy gene from CBA mice into the genome of AKR mouse genome [3,7]. The boundaries of this transferred fragment in the genome of AKR mice were labeled with two markers D13Mit74 (59 cM) and D13Mit214 (71 cM). Animal genotype was evaluated by PCR of DNA samples isolated from tail tip tissues using primers specific for D13Mit76, D13Mit74 и D13Mit214 markers [2]. AKR.CBA-D13Mit76 mouse strain was homozygous by CBA-allele D13Mit74 and by AKR-allele D13Mit214. CBA-allele of the gene encoding 5-HT_{1A} receptor localized 1 cM proximally (58 cM) to D13Mit74 marker was transferred to AKR.CBA-D13Mit76 mouse strain together with CBA allele of catalepsy gene.

All mice were at the age of 3 months, had body weight of 27±1 g, and after weaning were maintained in groups of 10 animals in 50×30×25-cm cages at natural illumination and temperature of 22±2°C. Complete ration and water were given *ad libitum*. The animals were maintained and used in experiments in according to the guidance of the Council of European Communities (Directive 86/309/EEC, November 24, 1986).

Two-three days before the experiment, the animals were placed into individual 50×30×25-cm cages to exclude the effects of group interactions. Selective agonist of serotonin 5-HT_{1A} receptors 8-OH-DPAT (Sigma) was dissolved in physiological saline, the acute effects of the drug (0.1, 0.5, and 1.0 mg/kg, intraperitoneally) was evaluated in tests of spontaneous intermale aggression and catalepsy. Controls received physiological saline.

For testing spontaneous intermale aggression, a mature outbred albino male mouse (intruder) was placed in a home cage of the test mouse (resident); the mice were comparable by the age and body weight. The test male mouse attacking the intruder for 10 min was considered as aggressive. The effect of 8-OH-DPAT on spontaneous aggression was evaluated by the percent of aggressive mice in each group [1].

Catalepsy was tested in CBA and AKR.CBA-D13Mit76 mice for 2 days. On day 1, animals exhibiting predisposition to cataleptic reaction were selected. Freezing was induced by a series of pinches on the back of the neck; the animals retaining the artificial posture for at least 20 sec in 3 of 10 successive tests were considered as cataleptic [2]. On day 2, catalepsy in cataleptic mice was induced by a series of 3 successive pinches before and 20 min after injection and the time of freezing after each pinch was recorded. The effect of 8-OH-DPAT was evaluated by comparing the total immobility time before and after administration of the preparation.

Spontaneous aggression in each group of each strain was expressed as the percent of aggressive mice and compared to that in the control group (two-way normal distribution) after conversion of percents into radians using Fisher transformation and Bonferroni correction for the number of comparisons (equal to 3). The total freezing time before and after drug injection was compared using repeated measures ANOVA followed by Fisher multiple comparison.

RESULTS

In all applied doses 8-OH-DPAT suppressed aggression in CBA mice ($p<0.05$), while in AKR and AKR. CBA-D13Mit76 mice the drug produced a significant inhibiting effect only in a dose of 1 mg/kg, although a 2-fold decrease in the relative number of aggressive animals was observed even after administration of 0.5 mg/kg 8-OH-DPAT (Table 1). Alleviation of catalepsy in CBA mice was noted only after administration of 0.5 and 1.0 mg/kg 8-OH-DPAT (Fig. 1, *a*); analysis of repeated measurements revealed significant effects of factor "time" ($F(1,36)=10.54$, $p<0.01$) and interaction of factors "drug" and "time" ($F(3,36)=10.29$, $p<0.001$), but not for factor "drug " ($F(3,36)=0.18$, $p>0.05$). At the same time, 8-OH-DPAT in all three doses significantly shortened the time of cataleptic freezing in AKR.CBA-D13Mit76 mice (Fig. 1, *b*), analysis of repeated measurements showed a significant effect of factor "time" ($F(1,34)=54.04$, $p<0.001$) and interaction of factors "drug" and "time" ($F(3,34)=7.87$, $p<0.001$); a tendency towards reliability of factor "drug" was also noted ($F(3,34)=2.37$, $p<0.09$).

Thus, 8-OH-DPAT in the low dose (0.1 mg/kg) primarily affecting presynaptic receptors suppressed aggressive behavior in CBA mice, but did not change the time of cataleptic freezing. On the contrary, acti-

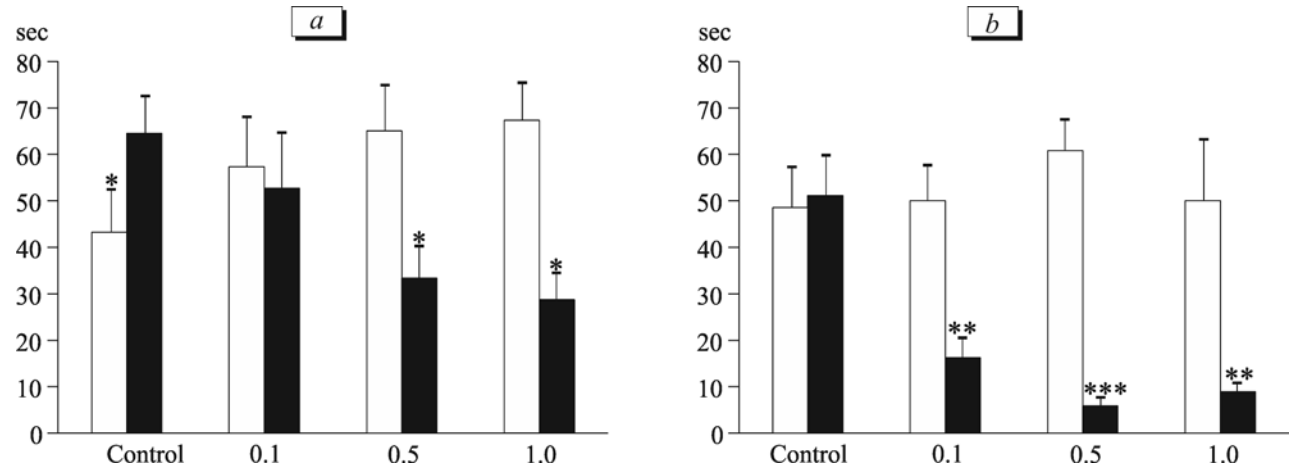

Fig. 1. Immobility time in the test for catalepsy in CBA (*a*) and AKR.CBA-D13Mit76 (*b*) mice before (light bars) and 20 min after (dark bars) administration of 8-OH-DPAT or physiological saline (control, *n*=8-12). **p*<0.05, ***p*<0.01, ****p*<0.001 compared to the control 20 min postinjection.

vation of 5-HT_{1A} autoreceptors had no effect on the relative number of aggressive male mice of AKR and AKR.CBA-D13Mit76 strains, but significantly shortened the duration of cataleptic freezing in AKR.CBA-D13Mit76 mice. High doses of 8-OH-DPAT (0.5 and 1 mg/kg) which affected mainly postsynaptic receptors inhibited catalepsy in CBA and AKR.CBA-D13Mit76 mice, and in a dose of 1 mg/kg suppressed aggression in all tested mouse strains.

The relative number of aggressive CBA mice receiving physiological saline was surprisingly low, despite 40% intact male CBA mice demonstrated aggression against the intruder [1]. It can be hypothesized that intraperitoneal injection is a very stressful procedure for these animals attenuates aggression and enhances alternative behavior (increase in the time of freezing in the catalepsy test, Fig. 1, *a*).

Since allele of the gene encoding 5-HT_{1A} receptor in AKR.CBA-D13Mit76 mice originates from the donor CBA strain, it can be hypothesized that the genome of the recipient AKR strain has a considerable effect on the involvement of this type of receptors into regulation of aggressive behavior and catalepsy. This result agrees with previous data on increased level of 5-HT_{1A} receptor mRNA in the midbrain of AKR.CBA-D13Mit76 mice compared to CBA mice, which is also determined by genetic factors localized in the genome of AKR mice [3].

Hence, pre- and postsynaptic 5-HT_{1A} receptors can be involved into regulation of both alternative strategies of defensive behavior, catalepsy and aggression. At the same time, molecular mechanisms underlying the effects of these receptors largely depend on the genetic background. Congenic AKR.CBA-D13Mit76 strain is a promising and interesting model for evaluation of molecular and genetic mechanisms of 5-HT_{1A} receptor participation in the regulation of defensive behavior.

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TABLE 1. Effect of 8-OH-DPAT on Intermale Aggression in CBA, AKR, and AKR.CBA-D13Mit76 Mice

Strain	Percent of aggressive animals, %			
	control	8-OH-DPAT dose, mg/kg		
		0.1	0.5	1.0
CBA	16.7 (30)	0* (16)	0* (12)	0* (14)
AKR	31.6 (19)	28.6 (14)	12.5 (16)	0** (13)
AKR.CBA-D13Mit76	63.2 (19)	66.7 (15)	29.4 (17)	7.6*** (13)

Note. Number of observations is shown in parentheses. **p*<0.05, ***p*<0.01, ****p*<0.001 compared to physiological saline.

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