GENETICS

Effect of Mouse Chromosome 13 Terminal Fragment on Liability to Catalepsy and Expression of Tryptophane Hydroxylase-2, Serotonin Transporter, and 5-HT_{1A} Receptor Genes in the Brain

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Congenic mice obtained by genome fragments transfer from one strain to another are a potent tool for studies of the molecular mechanisms of behavioral mutations. The 59-70 cM fragment of chromosome 13 containing the locus determining predisposition to freezing reaction (catalepsy) and the gene encoding 5-HT_{1A} receptor were transferred from cataleptic CBA/Lac mice into the genome of catalepsy-resistant AKR/J mice. The impact of this fragment for the severity of catalepsy and expression of genes encoding tryptophane hydroxylase-2, serotonin transporter, and 5-HT_{1A} receptor was studied. Half of mice of the resultant congenic AKR.CBA-D13Mit76 strain exhibited pronounced catalepsy, similarly to donor CBA animals. The expression of 5-HT_{1A} receptor gene in the midbrain of AKR animals was significantly higher than in CBA. The level of 5-HT_{1A} receptor mRNA in AKR.CBA-D13Mit76 animals was significantly higher than in the donor strain. Mice of parental AKR and CBA strains did not differ from each other and from AKR.CBA-D13Mit76 animals by the levels of tryptophane hydroxylase-2 and serotonin transporter genes mRNA. These data prove the location of catalepsy regulating gene in the distal fragment of chromosome 13. The recipient strain genome enhanced the expression of 5-HT_{1A} receptor gene in the brain without modulating the expression of catalepsy gene.

Key Words: *congenic mice; hereditary catalepsy; 5-HT_{1A} receptor gene; tryptophane hydroxylase-2; 5-HT-transporter*

Detection of the pathway from the gene to the sign, the sequence of molecular events through which gene mutations manifest by changes in behavior is the key problem of behavioral genetics. Congenic mice obtained by genome fragment transfer are the most potent models for studies of molecular mechanisms of behavioral mutations. We used congenic mice for studies of the role of the brain serotonin system in the molecular mechanisms of regulation of hereditary catalepsy in mice.

Catalepsy (tonic stupor, animal hypnosis, sham death) manifests as the state of long-lasting immobility accompanied by plastic muscular tone. The

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animal or human in a state of catalepsy can retain an imposed artificial uncomfortable posture for a long time. In birds and mammals, catalepsy is a passive defense freezing (hiding) reaction in response to appearance of a predator or other threatening stimuli [5]. Hypertrophic manifestations of catalepsy are symptoms of severe disorders of brain function [13].

There are many proofs in favor of the key role of the brain transmitter serotonin (5-HT) in the mechanism of hereditary catalepsy [12]. Serotonin is synthesized in 5-HT neurons in the midbrain, packed in vesicles, and delivered by the axonal transport to all structures of the brain. It is acknowledged that tryptophane hydroxylase-2 (TPH-2; determines the rate of 5-HT synthesis in the brain), 5-HT_{1A} receptor, and 5-HT transporter play the key role in the spatial and temporal regulation of 5-HT synapse function [4,9,15]. Changes in genes encoding these proteins are associated with hereditary behavioral disorders [10,14].

Hereditary catalepsy in GC rats (genetic catalepsy) is associated with high TPH-2 activity in the striatum [8] and low density of 5-HT_{1A} in the midbrain [11]. Agonists of 5-HT_{1A} receptor (8-OH-DPAT and flesinoxan) shorten the duration of cataleptic freezing in GC rats [6]. We previously located the gene determining liability to catalepsy in mice in the distal part of chromosome 13 near the gene encoding 5-HT_{1A} receptor [1].

The aim of this study was transfer of chromosome 13 fragment containing the allele determining high liability to catalepsy from CBA mice into the genome of catalepsy-resistant AKR mice and evaluation of the effect of this fragment on the severity of catalepsy and expression of genes encoding TPH-2, 5-HT transporter, and 5-HT_{1A} receptor in the midbrain.

MATERIALS AND METHODS

Experiments were carried out on adult male AKR. CBA-D13Mit76 (n=29), CBA/Lac (n=132), and AKR/J (n=29) mice. The CBA and AKR strains were maintained by close inbreeding for more than 40 years at Institute of Cytology and Genetics. The AKR.CBA-D13Mit76 strain was obtained by transfer of chromosome 13 fragment labeled with D13-Mit76 (61 cM) satellite linked with catalepsy gene from CBA mice to the genome of AKR mice. To this end, male (AKR×CBA)F_1 hybrids were mated with AKR females; heterozygous males were selected from back-crosses by D13Mit76 marker and mated with AKR females. The procedure was repeated 8 times. After 9 mating procedures, the heterozygous mice were mated between each other in order to obtain AKR.CBA-D13Mit76 animals with D13Mit76 CBA allele in AKR genome. The boundaries of this fragment of chromosome 13 transferred from CBA were detected in the AKR genome using two markers: D13Mit74 (59 cM) and D13-Mit214 (71 cM). Animal genotype was identified by PCR with primers specific for D13Mit76, D13-Mit74, and D13Mit214 markers using DNA samples isolated from tissues of the tip of the tail [1]. The AKR.CBA-D13Mit76 strain was homozygous by D13Mit74 CBA allele and by D13Mit214 AKR allele. Together with the CBA allele of catalepsy gene, CBA allele of 5-HT_{1A} receptor gene located 1 cM proximally (58 cM) from D13Mit74 marker was transferred to AKR.CBA-D13Mit76 strain (Fig. 1).

All animals were aged 2-3 months, weighing 25±2 g, kept in groups of 10 mice after separation from mothers in 50×30×25 cm cages at natural illumination, 22±2°C, food and water *ad libitum*. The animals were handled in accordance with the regulations of the European Community Council (directive 86/309/EEC of November 24, 1986).

In order to eliminate the group effect, the animals were isolated in individual cages (50×30×25 cm) 2-3 days before the experiment. Catalepsy was induced by a series of pinches of the nape of the neck. The percent of cataleptics retaining the posture which was imparted to them for at least 20 sec in no less than 3 of 10 tests was evaluated [1].

The levels of mRNA of TPH-2, 5-HT transporter, and 5-HT_{1A} receptor genes were measured in 10 mice of each strain. The animals were decapitated, the brain was placed on ice, and the midbrain was isolated. After location of the bodies of 5-HT neurons, the midbrain was rapidly frozen in liquid nitrogen and stored at -70°C.

Total RNA was isolated by extraction with a mixture of guanidine isothiocyanate, phenol, and chloroform. The levels of TPH-2, 5-HT transporter, and 5-HT_{1A} receptor gene mRNA were evaluated by RT-PCR using known concentrations of mouse genome DNA as the external standard [3]. The nucleotide sequences, primer annealing tempera-

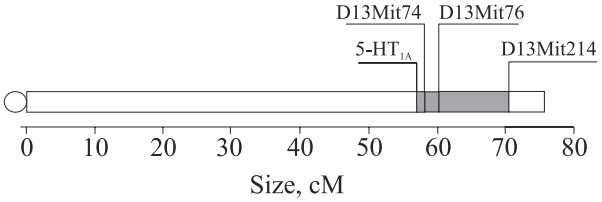

Fig. 1. Structure of AKR.CBA-D13Mit76 chromosome 13. Fragments from AKR and CBA are colored white and gray, respectively. The position of 5-HT_{1A} receptor gene and microsatellite markers is shown by vertical lines.

TABLE 1. Nucleotide Sequences and Characteristics of Primers

Gene	Nucleotide sequences	Annealing temperature, °C	Amplicon length, b. p.
5-HT_{1A} receptor	F 5'-gactgccaccctctgccctatatc	62	200
	5'-tcagcaaggcaaacaattccag		
TPH-2	F 5'-cattcctcgcacaattccagtcg	61	239
	R 5'-agtctacatccatcccaactgctg		
5-HT transporter	F 5'-aagccccaccttgactcctcc	57	198
	R 5'-ctccttcctctcctcacatatcc		
RNA polymerase 2	F 5'-gttgtcgggcagcagaatgtag	63	188
	R 5'-tcaatgagaccttctcgtcctcc		

tures, and amplicon sizes are presented in Table 1. No differences in RNA polymerase 2 gene mRNA in mice of the studied genotypes were detected ($F_{2.20}<1$, $p>0.05$), and therefore RNA polymerase 2 mRNA was used as the endogenous standard.

The level of mRNA was expressed by the number of copies of the studied gene per 100 copies of RNA polymerase 2 mRNA present in the samples (internal standard).

Liability to catalepsy was expressed as the percentage of cataleptics in the strain and compared using the χ^2 test. The levels of mRNA were compared using ANOVA with subsequent multiple comparison after Fisher.

RESULTS

Congenic AKR.CBA-D13Mit76 strain was derived as a result of 9 successive back crossings with AKR. The genome of the new strain was 99.8% AKR genome except a short fragment near D13-Mit76 marker (61 cM). The transfer of chromosome 13 fragment labeled with D13Mit76 microsatellite from CBA to AKR genome significantly modified the behavior of animals and transformed AKR mice not liable to catalepsy into cataleptic mice. This fact experimentally confirmed the location of the catalepsy gene in the transferred fragment of chromosome 13. It is noteworthy that sign penetrance in congenic mice well coincided with that in donor CBA mice: 51.7% cataleptics in the AKR.CBA-D13Mit76 strain and 54% in cataleptic CBA strain (Table 2). This fact proves that the genome of the recipient AKR strain did not appreciably modify the expression of catalepsy gene.

CBA allele of 5-HT_{1A} receptor gene was also transferred into AKR.CBA-D13Mit76 strain genome together with the catalepsy locus. The strains differed significantly by the concentrations of 5-HT_{1A} receptor mRNA in the midbrain (Table 3). The expression of 5-HT_{1A} receptor gene was minimum in CBA animals and maximum in AKR mice. This result is in good agreement with published data [2]. At the same time, the concentration of 5-HT_{1A} receptor mRNA in AKR.CBA-D13Mit76 mice (25.9±2.6) inheriting the 5-HT_{1A} receptor allele from CBA strain was equal to arithmetic mean of mRNA levels in parental CBA and AKR strains ((19.4± 31.5)/2=25.5). The increase in level of 5-HT_{1A} receptor mRNA in AKR.CBA-D13Mit76 mice ($p<0.1$ in comparison with that in donor strain CBA) indicates the effect of recipient (AKR) strain genome on the expression of this sign. It seems that this difference in the levels of 5-HT_{1A} receptor mRNA in the parental strains cannot be attributed exclusively to changes in the promoter of the corresponding gene. By contrast, genetic factors located in other parts of the genome are involved in the regulation of 5-HT_{1A} receptor gene expression. No differences in the levels of TPH-2 and 5-HT transporter genes mRNA in the midbrain were detected in mice of the three studied strains.

TABLE 2. Liability to Catalepsy in AKR, CBA, and AKR.CBA-D13Mit76 Mice

Strain	Number of animals	Number/% of cataleptics	χ^2 (p), df=1 (*vs.* AKR)
AKR	29	0 (0%)	
CBA	132	71 (54%)	27.9 ($p<0.001$)
AKR.CBA-D13Mit76	29	15 (51.7%)	20.2 ($p<0.001$)

TABLE 3. Levels of 5-HT_{1A} Receptor, 5-HT Transporter, and TPH-2 Gene mRNA in the Midbrain of AKR, CBA, and AKR.CBA-D13Mit76 Mice

Gene/structure	Strain			F(p)
	AKR	CBA	AKR.CBA-D13Mit76	
5-HT_{1A}	31.5±2.7 (8)	19.4±2.7* (9)	25.9±2.6^{+} (9)	$F_{2,22}$=5.0 (p<0.05)
5-HT transporter	100.7±15.5 (7)	119.1±14.5 (8)	85.8±14.5 (8)	$F_{2,20}$=1.3 (p>0.05)
TPH-2	64.5±13.6 (7)	56.9±12.7 (8)	44.4±12.7 (8)	$F_{2,20}$<1 (p>0.05)

Note. The data are presented as the number of the studied genes mRNA copies per 100 copies of RNA polymerase 2 mRNA. Figures in parentheses show the number of observations. *p<0.01 compared to AKR; ^{+}p<0.1 compared to CBA.

Despite the fact that our experiment failed to confirm an association between catalepsy and levels of TPH-2, 5-HT transporter, and 5-HT_{1A} receptor genes expression, this negative result does not contradict the key role of the brain 5-HT in the regulation of the freezing reaction. An association between high activity of TPH-2 and severity of hereditary catalepsy in rats [8] and mice [7] was previously demonstrated. Cataleptic CBA and ASC mice are characterized by higher sensitivity of 5-HT_{1A} receptor to 8-OH-DPAT agonist in comparison with noncataleptic AKR animals [2]. The catalepsy gene most likely regulates the function of these proteins at the posttranslational level. This conclusion is in good agreement with previous data indicating that the increase of TPH-2 activity in the striatum of cataleptic GC rats is a posttranslational event caused by local reversible phosphorylation of the enzyme [8].

Thus, transfer of chromosome 13 fragment from CBA to AKR mouse genome confirmed the location of catalepsy-regulating gene in the distal fragment of chromosome 13 and detected the effect of the recipient strain genome on the expression of 5-HT_{1A} receptor gene. The resultant congenic AKR.CBA-D13Mit76 strain is a prospective model for studies of molecular mechanisms of the realization of hereditary behavior.

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REFERENCES

1. A. V. Kulikov and D. V. Bazovkina, *Genetika*, **39**, 1066-1072 (2003).
2. V. S. Naumenko, E. M. Kondaurova, A. V. Kulikov, and N. K. Popova, *Dokl. Rossiisk. Akad. Nauk*, **409**, 133-135 (2006).
3. V. S. Naumenko and A. V. Kulikov, *Mol. Biol.*, **40**, 37-44 (2006).
4. N. M. Barnes and T. Sharp, *Neuropharmacology*, **38**, No. 8, 1083-1152 (1999).
5. A. K. Dixon, *Br. J. Med. Psychol.*, **71**, Pt. 4, 417-445 (1998).
6. A. V. Kulikov, V. G. Kolpakov, G. V. Maslova, *et al.*, *Psychopharmacology* (Berl.), **114**, No. 1, 172-174 (1994).
7. A. V. Kulikov, E. Y. Kozlachkova, N. N. Kudryavtseva, and N. K. Popova, *Pharmacol. Biochem. Behav.*, **50**, No. 3, 431-435 (1995).
8. A. V. Kulikov, E. Y. Kozlachkova, and N. K. Popova, *Ibid.*, **43**, No. 4, 999-1003 (1992).
9. K. P. Lesch, *Serotoninergic Neurons and 5-HT Receptors in the CNS*, Eds. H. G. Baumgarten, *et al.*, New York (1997), pp. 671-705.
10. N. K. Popova, *Bioessays*, **28**, No. 5, 495-503 (2006).
11. N. K. Popova, D. F. Augustinovich, V. G. Kolpakov, and I. Z. Plyusnina, *Pharmacol. Biochem. Behav.*, **59**, No. 4, 793-797 (1998).
12. N. K. Popova and A. V. Kulikov, *Am. J. Med. Genet.*, **60**, No. 3, 214-220 (1995).
13. B. Singerman and R. Raheja, *Ann. Clin. Psychiatry*, **6**, No. 4, 259-266 (1994).
14. J. Veenstra-VanderWeele, G. M. Anderson, and E. H. Cook Jr., *Eur. J. Pharmacol.*, **410**, Nos. 2-3, 165-181 (2000).
15. D. J. Walther, J. U. Peter, S. Bashammakh, *et al.*, *Science*, **299**, No. 5603, 76 (2003).