GENETICS

Effect of Chronic Administration of Imipramine on 2A-Serotonin Receptor mRNA in Brain Cortex of Rats Predisposed and Resistant to Catalepsy

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Rats selected by predisposition to catalepsy showed decreased level of 2A-serotonin receptor mRNA in the frontal cortex in comparison with Wistar rats ($p<0.05$). Chronic administration of tricyclic antidepressant imipramine hydrochloride 2-fold increased the content of receptor mRNA in genetically cataleptic rats ($p<0.001$) and did not change this parameter in Wistar rats. These results prompted us to revise current notion on the mechanisms of chronic effect of imipramine on 2A-serotonin receptors.

Key Words: *2A-serotonin receptors; imipramine; catalepsy; mRNA; brain cortex*

Long term administration of imipramine and other tricyclic antidepressants improve the state of depressive patients. Despite 50-year history of clinical application of imipramine, the mechanisms of its therapeutic effects remain unclear. Imipramine possesses high affinity to transport proteins responsible for removal of biogenic amines, serotonin and norepinephrine, from the synaptic cleft and limiting their interaction with the receptors on the postsynaptic membrane [3]. Imipramine blocks re-uptake of biogenic amines and prolongs their effects. Despite imipramine blocks re-uptake of biogenic amines as soon as after single administration, its therapeutic antidepressant effects are manifested only after long-term application [5]. The therapeutic effect of tricyclic antidepressants is determined by long-term changes in the density and sensitivity of serotonin (5-HT) receptors in the brain [4,10]. Decreased density of 2A-type 5-HT-receptor (5-HT_{2A}-receptor) in rat cortex after long-term administration of tricyclic antidepressants was demonstrated in experiments [6,8,13]. It is known that these preparations show no clinical activity on healthy subjects [14], therefore, the study of the effect tricyclic antidepressant on 5-HT-receptors in animals with genetically determined depressive behavioral changes combined with pronounced changes in the serotonin brain system is actual.

Genetically cataleptic (GC) rats were obtained by long-term selection of outbred Wistar rats for high predisposition to cataleptic freezing [1]. In contrast to Wistar rats, GC rats are characterized by prolonged freezing in catalepsy and forced swimming test [9] and decreased density of 5-HT_{2A}-receptors in the striatum and brain cortex [11].

The purpose of the present study was to examine the effect of imipramine on the expression of 5-HT_{2A}-receptor mRNA in the frontal cortex of GC and Wistar rats.

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MATERIALS AND METHODS

The experiments were carried out on adult male GC and Wistar rats (12 rats per group). At the beginning of the experiment the animals aged 2 months and weighed 180±12 g. The animals were kept separately in 60×40×20-cm cages throughout the experiment. Each strain was divided into 2 weight-matched groups. Control groups (6 animals of each strain) received water for 27 days, experimental animals (6 animals of each strain) received imipramine in water (daily dose 15 mg/kg). Imipramine dose and concentration were corrected for each rat depending on daily water consumption. Imipramine-containing water was changed every 24 h. After 24 days imipramine solution was replaced with tap water to decrease imipramine concentration in the body. After 72 h the animals were decapitated, the brain was promptly removed on cold, the frontal cortex was isolated, frozen in liquid nitrogen, and stored at -65°C until RNA isolation.

Total RNA was extracted with guanidine isothiocyanate and phenol [7]. The absence of DNA traces in RNA preparations was verified by PCR with β-actin primers. The content of 5-HT_{2A}-receptor mRNA was determined by reverse transcription-PCR presenting a modification of the method described elsewhere [2]. For reverse transcription 1 μg total RNA, 180 ng random primer, and 16 μl sterile 2.25 μmol KCl were mixed in a 0.5 ml tube, denaturated at 94°C for 5 min, and annealed at 41°C. A buffer (15 μl) containing 200 U M-MulV reverse transcriptase (Biosan), 0.225 μmol Tris-HCl (pH 8.1-8.3), 0.015 μmol each dNTP, 0.225 μmol dithiothreitol, and 0.03 μmol $MnCl_2$ was added to the tube. The mixture (final volume 31 μl) was incubated at 41°C for 60 min. The obtained cDNA was stored at -20°C. An aliquote (1 μl) was mixed with 2.5 μl mixture of 5-HT_{2A}-receptor gene-specific primers (5'-TGCAGAATGCCACCAACTT and 5'-TGCCAC AAAAGAGCCTATGAG, 5 pmol each) and 14 μl buffer containing 0.8 U Taq-polymerase (Medigen), 1 μmol KCl, 0.2 μmol Tris-HCl (pH 9.0), 0.004 μmol dNTP, 0.15% Triton X-100, and 0.03 μmol $MgCl_2$ (final volume 17.5 μl). The mixture was denaturated at 94°C for 4 min and amplified (10 cycles: 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C). β-Actin primers (5'-GGGAACCGCTCATTGCC and 5'-ACCCA CACTGTGCCCATCTA, 2.5 μl, 5 pmol each) were added to tubes, denaturated at 94°C for 4 min, and amplified in additional 25 cycles. Preliminary experiments under the same conditions revealed linear amplification of β-actin and 5-HT_{2A}-receptor gene fragments within 27 and 38 cycles, respectively. Primers for 5-HT_{2A}-receptor gene amplification were selected for gene exons 1 and 3 so that they were separated by several thousands base pairs (b.p.) and tested for specificity by Fasta3 database. The correspondence between the nucleotide sequence of 413 b.p. amplification product and the fragment of 5-HT_{2A}-receptor mRNA was confirmed by restriction analysis with Alu I. The fragment of β-actin gene was amplified using conventional primers [15]. Amplification products (15 μl) were mixed with 5 μl 0.35% orange G in 30% sucrose and analyzed by electrophoresis in 1% agarose with 0.5x TBE buffer. After ethidium bromide staining, the gels were scanned in UV and band intensity was determined with the help of Scion Image program (Scion Corporation). The expression of 5-HT_{2A}-receptor mRNA was evaluated as the ratio between band intensities corresponding to 5-HT_{2A}-receptors and β-actin PCR products.

The data were processed statistically and the significance of differences was evaluated by ANOVA and Dunkan test.

RESULTS

Significant differences in the initial levels of 5-HT_{2A}-receptor mRNA expression in Wistar and GC rats ($F_{1.10}$=7.1, p<0.03; Table 1) were found. Significant effects of the factors "influence" ($F_{1.20}$=7.1, p<0.02) and interaction between factors "strain" and "influence" ($F_{1.20}$=13.5, p<0.002) were revealed. Chronic imipramine administration had no effects on the expression of 5-HT_{2A}-receptor mRNA in rat cortex (p>0.05), but 2-fold increased the content of 5-HT_{2A}-receptors in the frontal cortex of GC rats (p<0.001).

Decreased content of 5-HT_{2A}-receptor mRNA in the cortex of GC rats compared to that in Wistar rats agrees with previous data on decreased functional activity and density of these receptors in the brain of GC rats [11]. It can be assumed that changes at the level of regulation of 5-HT_{2A}-receptor gene expression underlie genetically determined receptor desensitization in GC rats.

Chronic imipramine administration produced different effects on the content of 5-HT_{2A}-receptor mRNA in Wistar and GC rats. In Wistar rats imipramine did not change the content of 5-HT_{2A}-receptor mRNA, whereas in GC rats it 2-fold increased this

TABLE 1. Effect of Imipramine on Expression of 5-HT_{2A}-Receptor mRNA in Frontal Cortex of Wistar and GC Rats (M±m, n=6)

Rats	Control	Imipramine
Wistar	2.19±0.10	1.91±0.32
GC	1.73±0.12*	3.44±0.39+

Note. *p<0.05 compared to control Wistar rats; +p<0.001 compared to the corresponding control.

parameter. This seems contradictory, because many authors showed decreased density of 5-HT_{2A}-receptors in the cortex after several weeks of imipramine administration [6,8,13], and decreased content of receptor mRNA could be expected. However, the absence of changes in the content of 5-HT_{2A}-receptor mRNA in Wistar rats treated with imipramine observed in our experiment agrees with published data that imipramine has no effect on 5-HT_{2A}-receptor mRNA in the cortex of normal animals [6,12]. It can be assumed that at least in Wistar rats, chronic administration of imipramine causes receptor desensitization on the post-translational, but not on the gene level. The sharp imipramine-induced increase in the expression of 5-HT_{2A}-receptor mRNA in GC rats attests to peculiarities of this strain and demonstrates the effect of imipramine on 5-HT_{2A}-receptor regulation at the genome level. The fact that enhanced mRNA receptor expression in GC rats was observed 72 h after termination of imipramine treatment suggests that this increase is associated with stable and long-term changes in the regulation of 5-HT_{2A}-receptor gene. Our findings indicate that current notion on the mechanisms of chronic effects of imipramine on 5-HT_{2A}-receptors has to be revised. GC rats can serve as a new model for the study of the molecular mechanisms underlying the therapeutic effect of imipramine and other antidepressants.

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