INTRODUCTION

The gp130 glycoprotein is encoded by \textit{Il6st} located on human chromosome 5 and mouse chromosome 13. It is a receptor component for a range of important cytokines, including interleukines 6, 11, and 27, oncostatin M, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin 1, cardiotrophin-like cytokine, and neurotrophin [1–3]. The formation of the ligand-receptor subunit gp130 complex activates cytosol Janus kinases which phosphorylate gp130 and the transcription factor STAT. Phosphorylated STAT undergoes dimerization and interacts with target DNA domains. This chain of events activates neuronal expression of the genes encoding the glial protein GFAP [4–8], TUBB3 and Krox 20 enolases [7], α-internexin, synapsins I and II [4], neurokinin 1 receptor [9], μ-opioid receptor [10], and A\textsubscript{1} adenosine receptor [11].

As a component of the interleukin-6 (IL-6) receptor, gp130 is involved in nonspecific immune response to bacterial infection. Administration of lipopolysaccharide (LPS), a toxic component of the Gram-negative bacterial cell wall, activates IL-1, IL-6, and TNF secretion [12]; it is them that cause sickness and depression-like behavior: decreased motor activity and sucrose consumption (anhedonia). They also increase the immobility time in the forced swim test in mice [13].

It has been proposed that behavioral effects of LPS and IL-6 are associated with their influence of the serotonin (5-hydroxytryptamine, 5-HT) mediator system of the brain [14], which comprises a group of neurons that produce 5-HT and use it as the main mediator. 5-HT is synthesized from \textit{L}-tryptophan, an essential amino acid. The rate-limiting step of the 5-HT synthesis is the \textit{L}-tryptophan hydroxylation to 5-oxytryptophan catalyzed by tryptophan hydroxylase 2 [15, 16]. The mediator is deposited in granules, accumulated in synaptic terminals, and secreted into the synaptic cleft at depolarization of 5-HT neurons. 5-HT secretion is subject to feedback regulation by presynaptic 5-HT\textsubscript{1A} receptors located on the bodies of 5-HT neurons [17]. The secreted mediator regulates behav-
The objective of this study was to investigate the role of gp130 in the molecular regulation of the 5-HT system of the mouse brain. This included the following tasks: to compare the levels of mRNAs encoding TPH2, 5-HT transporter, and 5-HT1A receptors and to analyze the LPS effect on the expression of these genes in the brain of AKR and AKR.CBA-D13Mit76 mice.

EXPERIMENTAL

Animals. The study was performed using adult male mice (age, 3–4 months; weight, 28 ± 0.5 g) of the strains AKR/J and AKR.CBA-D13Mit76. The strain AKR has been sustained by strict inbreeding for over 20 years. The strain AKR.CBA-D13Mit76 has been derived by introducing the D13Mit76-marked fragment of chromosome 13 from the strain CBA/Lac into the AKR/J genome by nine backcrosses to AKR. This congenic strain is also sustained by strict inbreeding. AKR.CBA-D13Mit76 differs from AKR in the 55–70 cM fragment of chromosome 13 derived from the CBA strain [26]. The fragment flanked with D13Mit74 and D13Mit214 microsatellites contains the gp130- and 5-HT1A receptor-encoding genes (Fig. 1). Animals were weaned at the age of four weeks, segregated by sex, and kept in cages sized 50 × 30 × 25 cm, six animals per cage, in standard conditions (air temperature, 22 ± 2°C, relative humidity 65%, and natural illumination). Animals were supplied with complete feed and water ad libitum. The maintenance and experimental procedures followed the guidelines of the European Community Council (Directive 86/609/EEC of November 24, 1986).

To remove the group interaction effects, animals were put into individual cages of the same size 2–3 days before the experiment. Mice of either line were divided in two weight–balanced groups. Animals of the control group were injected with saline and those of the test group were injected with LPS saline solution (E. coli 055 : B5, Sigma, the United States, 50 μg/kg ip). Preliminary experiments had shown that administration of 50 μg/kg LPS altered the behavior of AKR.CBA-D13Mit76 but not of AKR mice. Animals were decapitated 3 h after LPS administration; the brain was rapidly removed in the cold; the frontal cortex, the hippocampus, and the midbrain were isolated, frozen in liquid nitrogen and stored at −70°C.

RT–PCR. Total RNA was isolated using the standard guanidine thiocyanate–phenol–chloroform procedure [29] and dissolved in DEPC-treated water. The probes were purified from genomic DNA with DNase I (Promega, the United States), and the concentration was determined by optical density. RNA was diluted with sterile water to 0.125 μg/μl, and stored at −70°C. Contamination of RNA probes with genomic DNA was controlled by PCR with primers to the β-actin gene (Table) [30]. cDNA was synthesized using 8 μl (1 μg) RNA aliquots, random hexamer primers and 200 units reverse transcriptase (Biosan, Russia). The obtained cDNA probes were stored at −20°C. Contamination of cDNA probes with genomic DNA was detected using PCR with primers to exons 5 and 6 of the mouse tryptophan hydroxylase 1 gene (Table), which is not expressed in the brain [29]. In all the probes studied, the concentration of genomic DNA was less than 80 copies per μl.

Levels of mRNAs encoding TPH2, 5-HT transporter, and 5-HT1A- and 5-HT2A receptors were determined using quantitative PCR [29]. Mouse genomic DNA (strain C57BL/6J) in known concentrations and RNA polymerase II cDNA were used as the external and internal standard, respectively. For primer sequences, annealing temperature, cycle numbers, and amplicon size, see the table. The mRNA levels for the genes in question were expressed as the copy numbers per 100 molecules of RNA polymerase II mRNA [30, 31].

The 5-HT1A- and 5-HT2A receptor mRNA levels were determined in all three brain structures. The levels of TPH2 and 5-HT transporter mRNAs were investigated in the midbrain only, since the expression of these genes is restricted to serotonergic neurons.

Statistical analysis. For each gene, mRNA levels in each group were presented as mean ± standard error.

**Fig. 1.** The structure of the chromosome 13 fragment transferred from the CBA strain into the AKR genome to produce the congenic strain AKR.CBA-D13Mit76. Shown are the positions of Il6st, 5-HT1A receptor gene, and microsatellite markers.