PHARMACOLOGY AND TOXICOLOGY

Effect of Acute Alcohol Administration on Cerebral Content of Tyrosine Hydroxylase mRNA in Mice with Different Ethanol Sensitivity

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The levels of tyrosine hydroxylase mRNA in different brain regions of inbred mice with different sensitivity to alcohol were evaluated. This parameter was also measured 2, 4, and 6 h after single intraperitoneal injection of ethanol. We revealed interstrain differences in the expression of tyrosine hydroxylase gene in the cerebral cortex, hypothalamus, and *locus ceruleus*. Single ethanol injection caused different changes in tyrosine hydroxylase gene expression and reduced the interstrain differences. We conclude that the system of long-term regulation of tyrosine hydroxylase gene is involved in the mechanisms of congenital alcohol sensitivity.

Key Words: *ethanol; tyrosine hydroxylase; gene expression; inbred lines*

Changes in activity of the catecholamine and, especially, dopamine neurotransmitter systems in the development and maintenance of drug and alcohol abuse are well studied [4,10]. Significant disturbances in the cerebral catecholamine system were revealed both in modeled drug abuse and in alcoholic patients [2,5].

Our previous experiments showed that [1] enhanced dopamine (DA) metabolism in cerebral structures functionally related to the reinforcement system provide neurochemical bases for drug abuse. Psychoactive drugs significantly increase DA concentration due to its intense release from intracellular stores and inhibition of its reuptake from synaptic cleft. Chronic treatment with psychoactive drugs depletes DA stores and stimulates its *de novo* synthesis by tyrosine hydroxylase (TH, tyrosine monooxygenase, E.C. 1.14.16.1). TH is the key enzyme of catecholamine synthesis transforming tyrosine into DA precursor DOPA and determining functioning of the whole catecholamine system.

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In vivo and *in vitro* experiments showed that ethanol modulates enzyme activity [6,11-14]; however, its effect on TH is little studied.

Insufficiency of immediate compensatory reactions of DA synthesis regulation under conditions of chronic influence of psychoactive drugs (in particular, ethanol) activates long-term regulatory mechanisms. For instance, TH synthesis increased due to enhanced transcription of TH gene thus maintaining DA metabolism at a higher level. This new level of DA metabolism probably manifests established alcohol abuse.

It is known that long-term alcohol consumption does not necessary results in alcohol dependence and the risk of alcoholism development does not correlate with the initial alcoholic motivation. It is possible that individual risk of alcoholization depends on long-term regulation of enzyme activity (in particular, TH activity) at the genome level.

We assumed that ethanol modulates long-term TH regulation and changes TH gene transcription in neurons, and that alcoholization reveals individual genetically determined peculiarities in the regulation of TH gene.

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Our previous studies demonstrated significant differences in TH gene transcription in the mesencephalon and cortex of experimental animals preferring and rejecting alcohol after 9-month alcoholization [3]. We also showed differences in TH gene expression in the mesocorticolimbic and nigrostriatal dopamine systems in mice with different ethanol sensitivity.

The aim of the present study was to evaluate TH gene expression in the brain of two inbred mouse strains characterized by different ethanol sensitivity and analyzed the dynamics of TH gene expression after single ethanol dose.

MATERIALS AND METHODS

Experiments were carried out on HAFT (n=20) and LAFT (n=20) mice characterized by high and low tolerance to ethanol, respectively. The selection procedure consisted in determining the time of recovery of inclined plain walking after second ethanol dose. Mean recovery time was 90 and 130 min for HAFT and LAFT mice, respectively. This allowed to specify LAFT and HAFT mice as more and less sensitive to ethanol, respectively.

Mice previously never receiving ethanol were injected intraperitoneally with 15% (3.75 g/kg) ethanol in physiological saline. Control mice (n=5 from each strain) received the same volume of physiological saline.

The animals were decapitated 2, 4, and 6 h postinjection (5 animals per point).

TH gene expression in the frontal cortex, hypothalamus, striatum, and *locus ceruleus* was evaluated by Northern-blot analysis on frozen specimens.

Total RNA fraction was isolated by guanidine thiocyanate method with phenol-chloroform extraction [8]. Total RNA (15 μl) was fractionated by horizontal electrophoresis. Electrophoregrams were transferred to HYBOND-N membranes (Amersham) for 12-14 h. Membrane hybridization was based on incorporation of labeled probes in TH mRNA. Synthetic Russian-made oligonucleotide complimentary to TH mRNA region was used as the probe. Radioactive label (α-32P ATP) was enzymatically bound to oligonucleotide using terminal transferase. After hybridization the membranes were washed, dried, and exposed with x-ray film for 16-72 h. mRNA concentration was determined by measuring relative optical density of exposed regions on the film corresponding to the presence of TH mRNA-bound radioactive probe on a 2202 ULTROSCAN scanning densitometer (LKB).

The data were processed statistically using Student's t test for independent variables

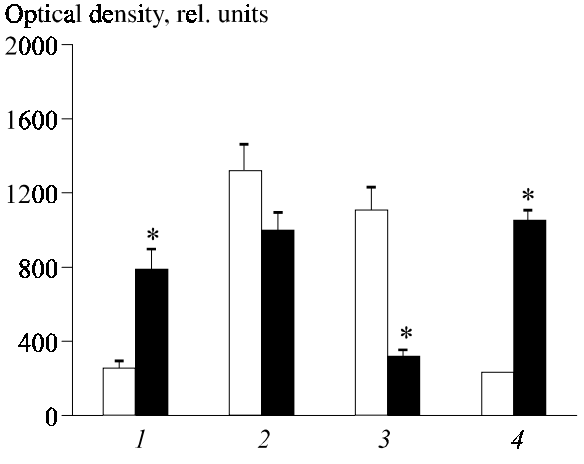

Fig. 1. Tyrosine hydroxylase mRNA in the frontal cortex (*1*), striatum (*2*), hypothalamus (*3*), and *locus ceruleus* (*4*) of LAFT (open bars) and HAFT (dark bars) mice. *p<0.05 compared to LAFT mice.

RESULTS

In the frontal cortex the baseline level of TH mRNA in control LAFT mice 3-fold surpassed that in HAFT mice (p<0.05, Fig. 1). These interstrain differences disappeared after ethanol injection.

Two, 4, and 6 h after acute ethanol administration the content of TH mRNA in LAFT mice significantly decreased to 37, 14, and 24% of the control, respectively (p<0.05, Fig. 2, *a*). In HAFT mice, acute ethanol injection had practically no effect on TH mRNA level. No interstrain differences were observed throughout the experiment.

In the striatum TH gene expression was similar in two mouse strains. Two, 4, and 6 h after ethanol injection striatal TH mRNA level in LAFT mice decreased to 15, 19, and 11% of the control, respectively (p<0.05, Fig. 2, *b*). In HAFT mice, this parameter showed decreased insignificantly. No interstrain differences after ethanol injection were found.

In the hypothalamus the initial level of TH mRNA in LAFT mice was 3 times lower than in HAFT mice (p<0.05, Fig. 1). These interstrain differences disappeared after ethanol injection. Four and 6 h postinjection TH mRNA levels in LAFT mice decreased to 13 (p<0.01) and 40% of the control, respectively (Fig. 2, *c*).

Interestingly, in HAFT mice ethanol injection induced TH mRNA response only in the hypothalamus: 4 and 6 h postinjection the content of TH mRNA decreased to 10 and 21% of control, respectively, (p<0.05). These opposite changes in TH mRNA levels abolished the interstrain differences in this parameter.

The content of TH mRNA in *locus ceruleus* of the control LAFT mice 3.5-fold surpassed that of the control HAFT mice (p<0.05, Fig. 1). Two hours postinjection TH mRNA level in LAFT mice decreased to 34% of the control, while interstrain differences dis-

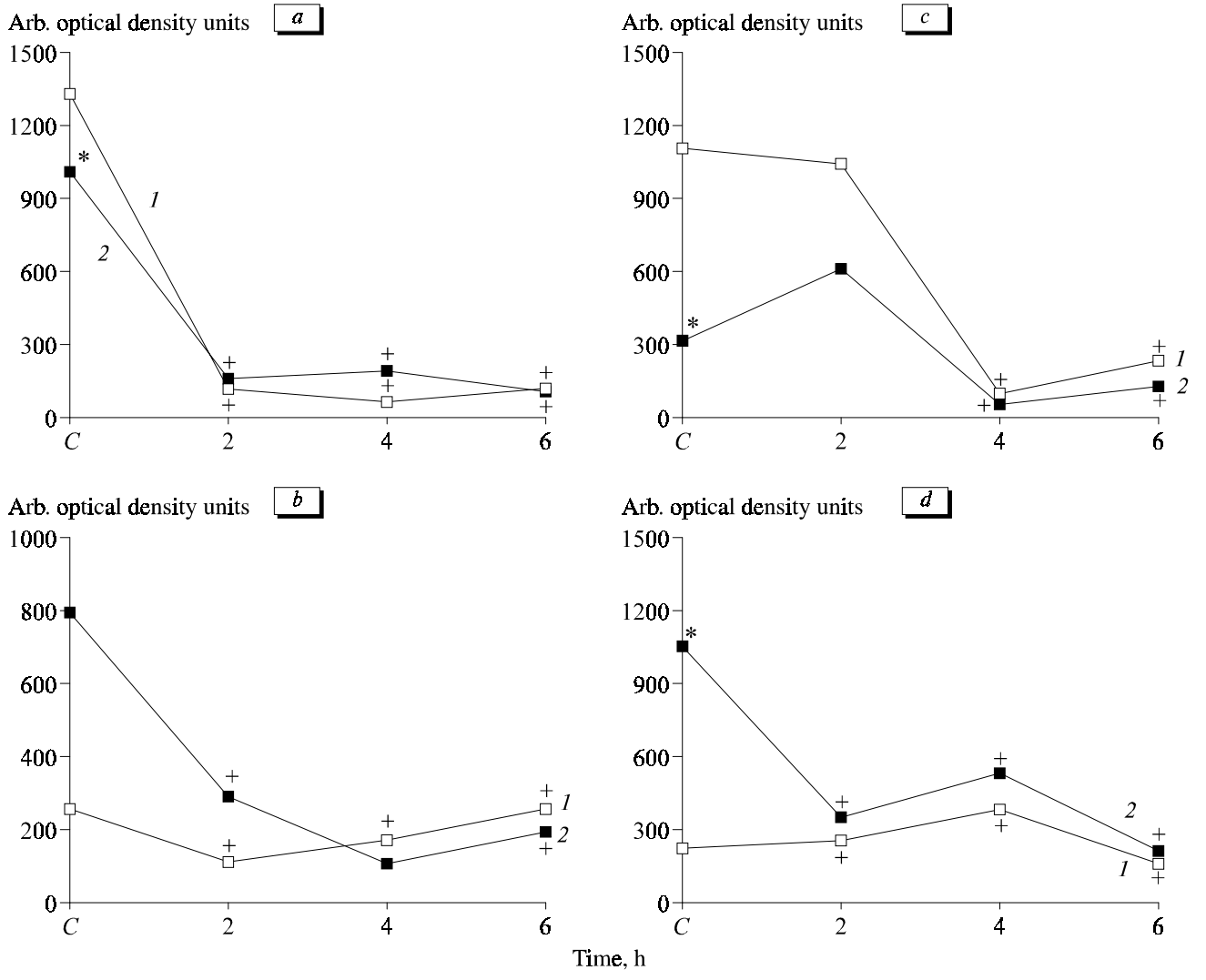

Fig. 2. Dynamics of tyrosine hydroxylase mRNA levels in the frontal cortex (*a*), striatum (*b*), hypothalamus (*c*), and *locus ceruleus* (*d*) of LAFT (*1*) and HAFT (*2*) mice after acute ethanol injection. $p<0.05$ *compared to LAFT or $^{+}$control mice.

appeared. Four and 6 h postinjection TH mRNA levels were 49 and 25% of the control, respectively ($p<0.05$, Fig. *d*). Acute ethanol injection had no effect on TH mRNA in HAFT mice, but eliminated the interstrain differences at all terms of the experiment.

Thus, inbred mice with different ethanol sensitivity showed a correlation between behavioral parameters and TH mRNA levels in the mesolimbic dopamine system. At the neurochemical level, high ethanol sensitivity corresponded to a higher initial level of TH gene expression. Single ethanol injection significantly decreased this parameter in highly sensitive animals to a level typical of animals with low ethanol sensitivity.

It should be noted that single ethanol injection had no effect on TH gene expression in the dopamine neurotransmitter system in animals with low ethanol sensitivity.

These findings suggest that ethanol modulates TH gene expression and that the reactions of TH gene transcription system to ethanol is genetically determined. This confirms the presence of congenital biological factors determining different sensitivity to ethanol.

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