PHARMACOLOGY AND TOXICOLOGY

Effect of Ethanol Consumption on the Expression of the Tyrosine Hydroxylase Gene in Rat Brain and Adrenals

I. P. Anokhina and A. O. Kibitov

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After chronic alcoholization for 9 months with increased doses of ethanol, the tyrosine hydroxylase gene in the brain and adrenals of rats is expressed at different levels depending on the intensity of the desire for alcohol.

Key Words: *ethanol; alcohol dependence; tyrosine hydroxylase; gene expression*

Results of experimental and clinical studies led us to conclusion that a leading role in the establishment of alcohol dependence is played by functional changes in the catecholamine neurotransmitter system in the brain. Enzymes, particularly monoamine oxidase, dopamine-β-hydroxylase, tyrosine hydroxylase, and catechol-ortho-methyltransferase, play an important role in the regulation of catecholamine metabolism. There is a large body of evidence that the activities of these enzymes are altered during prolonged alcoholization [1,3,5], and some of these changes may be implicated in the development of alcohol dependence [3,6-8].

Tyrosine hydroxylase (TH), the key enzyme in catecholamine synthesis, converts the amino acid tyrosine into the dopamine precursor dihydroxyphenylalanine, and alterations in the enzyme activity, particularly if sustained, may strongly determine the function of the catecholamine system. However, the neurochemical nature of shifts in the activity of enzymes involved in catecholamine metabolism during prolonged alcoholization has not been ascertained. These shifts may be caused by changes in the transcriptional activity of genes coding for the enzyme.

Alcohol dependence does not necessarily result from long-lasting alcohol consumption and, moreover, the risk of alcoholism does not correlate with the level of initial alcohol motivation. Presumably, individual risk of alcoholism is linked to a long-term regulation of the activities of catecholamine-metabolizing enzymes, including TH, at the level of the nerve cell genome.

The objectives of the present study were to evaluate the effect of chronic alcohol intoxication on the expression of the TH gene in the brain and adrenals of experimental animals and to examine changes in the expression of this gene in these organs in relation to the degree of pathological desire for alcohol.

MATERIALS AND METHODS

Male Wistar rats aged 3 months were used. They received ethanol in increasing concentrations as the only source of liquid: 5% ethanol solution during the 1st week, 10% solution during the 2nd, and 15% solution during the 3rd week and, until the end of the 9-month period of alcoholization. Then the animals had free choice between water and 15% ethanol solution for 2 weeks. Ethanol consumption during these 2 weeks was calculated from the following formula: $C = V_{et}/V_{li}$, where V_{et} is the volume of 15% ethanol consumed per day and V_{li} is the total volume of liquid consumed per day. Ethanol consumption for each rat was calculated as the mean of daily C values over the 2-week period.

Laboratory of Psychopharmacology, Institute of Narcology, State Research Center for Psychiatry and Narcology, Ministry of Health of the Russian Federation, Moscow

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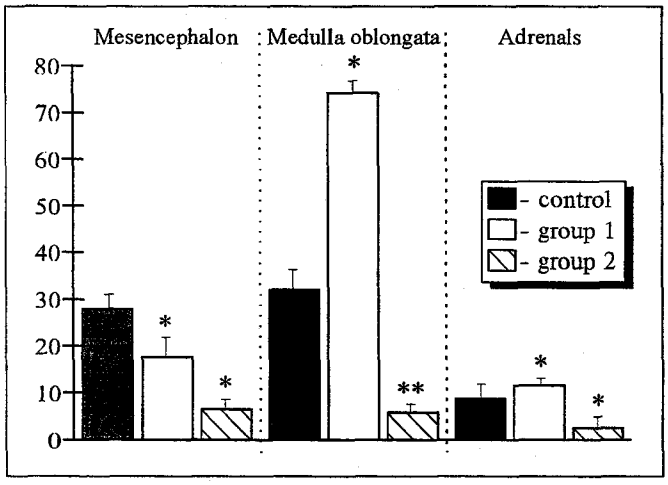

Fig. 1. Expression of the tyrosine hydroxylase gene in the brain and adrenals of rats that had consumed ethanol at different levels for 9 months. Ordinate: optical density (rel. units). *$p<0.05$, **$p<0.01$ in comparison with the control group.

For further experiments, rats with the lowest and highest C values were assigned into two groups differing in the level of ethanol consumption when allowed to choose freely between ethanol and water: group 1 (n=5) with $C>0.5$ (50%) and group 2 (n=4) with $C<0.1$ (10%). The control group consisted of 5 rats maintained for 9 months under the same conditions but were not alcoholized.

The expression of the TH gene was determined by measuring the concentration of TH mRNA with the use of Northern blot analysis.

After experiment the rats were decapitated. The mesencephalon, medulla oblongata, and adrenals were collected and frozen in liquid nitrogen. Total RNA was isolated by the guanidine thiocyanate method with phenol-chloroform extraction [4]. Total RNA aliquots (15 μl) were separated by horizontal electrophoresis, and the electrophoregrams were used for RNA blotting by capillary diffusion onto HYBOND-N membranes (Amersham) for 12-14 h. Membrane hybridization involved incorporation of a radiolabeled probe into the TH mRNA. The probe was an oligonucleotide complementary for the sequence of nucleotide pairs in the mRNA region for TH. The radioactive label (α-^{32}P-ATP) was inserted into the probe by enzyme reaction with terminal transferase (Fermentas). Parallel hybridization with β-actin was performed to control nonspecific binding. After hybridization the membranes were washed, dried, and exposed on an X-ray film for 16-72 h. The mRNA concentration was determined estimated in an Ultroscan-2202 scanning laser densitometer (LKB) by measuring the relative optical densities of the bands corresponding to the sites of the TH mRNA-bound radioactive probe was present. The intergroup differences were analyzed by standard Student's t test for unrelated variables.

RESULTS

The TH mRNA levels differed significantly in experimental and control rats (Fig. 1). In group 1 (high ethanol consumption), the level of TH gene expression was 4 times lower in the mesencephalon ($p<0.05$) and 6 times lower in the medulla oblongata ($p<0.01$). In group 2 (rats with low ethanol consumption), it was 1.5 times lower in the mesencephalon ($p<0.05$) but 2.5 times higher in the medulla oblongata ($p<0.05$) compared with the control group (Fig. 1).

The intergroup differences were observed in the TH gene expression in the adrenals. In comparison with the control group, the concentration of TH mRNA in group 1 was 3 times lower ($p<0.05$), i.e., the differences between these groups were comparable to those for the brain; in group 2, the mRNA level was 25% higher ($p<0.05$).

These are preliminary and prompt experiments. However, it is clear that prolonged alcoholization strongly influences the mechanisms of long-term TH regulation by altering the transcriptional activity of the TH gene in the brain and adrenals. Since this effect was much greater in the brain structures than in the adrenals, the action of ethanol on the transcriptional apparatus of brain neurons appears to be more specific. Our findings show that chronic ethanol consumption influences the genetic apparatus of neurons, suggesting that at least some functional changes in the catecholamine neurotransmitter system are due to the effect of alcohol on genetic regulation of TH activity.

On the other hand, the correlation of changes in the TH gene expression with the intensity of pathological desire for alcohol in animals suggests that changes in the long-term regulation of TH activity at the neuronal genome level are implicated in the mechanism by which alcohol dependence is established. It is noteworthy that the level of the TH gene expression was higher in the medulla oblongata and adrenals of rats with higher resistance to alcohol, i.e., the response in these rats was opposite to that observed in rats with alcohol dependence. This may indicate that individual response of the nerve cell to chronic alcohol intoxication is genetically determined.

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Changes in Neurons of the Caudate Nucleus in Experimental Alcoholism

E. N. Popova

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Reversible changes in caudate neurons and their dendrites in line with the components of compensatory reaction in oligodendrocytes are observed in rats during the first 3 weeks of a 12-month alcoholization period. After 2-4 months of alcoholization (the development of dependency), degenerative changes occur in caudate neurons and their dendrites system were evident. By the end of the 12-month period of alcohol intoxication, intensified deafferentation of the dendritic system was observed, suggesting functional insufficiency of the caudate nucleus.

Key Words: *alcohol; caudate nucleus; neurons; dendrites*

Morphofunctional organization of the caudate nucleus (CN) can be regarded as a subcortical association center involved in the regulation of brain integrative activity [6,10,11,13].

It was shown that CN is involved in neurophysiological mechanisms underlying the development of alcoholism and associated extrapyramidal disturbances [2]. However, structural organization of CN in chronic alcohol intoxication has been poorly investigated. In guinea pigs given small doses of alcohol over a short period, changes in neurons were detected not only in the neocortex but also in the CN [5]. Swollen and shrunken cells and occasional ghost cells were observed in CN of rats receiving alcohol for 1-3 months, while most CN neurons were swollen in rats after 6-12 months of alcohol consumption [3]. As we are aware, changes occurring in dendrites and dendritic spikes of experimentally alcoholized animals have not been studied, although these structures play an important role in primary processing of information received by the neuron and in the synaptic mechanisms of brain activity.

In the present study we examined changes in caudate neurons and in their dendrites with large numbers of spikes occurring in chronic alcohol intoxication (12 months).

MATERIALS AND METHODS

Rat model of chronic alcohol intoxication (alcoholization) was developed at the Department of Higher Nervous Activity, Moscow State University [7].

Rats were given a 30% alcohol solution instead of water from specially designed drinking bowls containing sucrose solution (150 g/liter) to increase alcohol consumption. The standard chow was supplemented with penicillin (daily dose 10,000 U) and polyvitamins (0.1-0.15 g). Control rats received an alcohol-free ration. Daily alcohol consumption was 2-3 ml per rat during the first 10-20 days, rose to 8-10 ml over the next 2-2.5 months and then to 14-17

Laboratory of Brain Ultrastructure, Institute of Brain, Russian Academy of Medical Sciences, Moscow

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