TYROSINE HYDROXYLASE ACTIVITY AND GENE EXPRESSION IN MICE WITH CONTRASTING DOMINATING POWER DURING SOCIAL STRESS

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The study of the neurochemical and molecular bases of integrative brain activity during adaptation of animals to external environmental conditions is an urgent task in neurobiology and neurogenetics. In the course of formation of hierarchic relations in a population, fundamental differences in changes in brain levels of noradrenalin and dopamine have been discovered [6] in mice with dominant and subordinate types of behavior. Meanwhile the catecholamine concentrations do not always reflect the neurochemical processes taking place in the neurotransmitter systems of the brain in response to environmental action. For instance, no information could be found in the literature on the study of activity of tyrosine hydroxylase, a key enzyme of catecholamine biosynthesis, or the functional activity of its gene in mice during social stress. The present investigation was devoted to the study of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on mature male A/He, CBA/Lac, C57BL/6j, DD, YT, and PT lines, weighing 22-25 g. Before the beginning of the experiment the animals were kept one in a cage for 5 days. Groups of six males, one of each genotype, were formed. Throughout the experiment, visual observations were made regularly 3 times a day, for 20 min each time, during the morning; during these observations different elements of the animals' behavior were recorded and dominant and subordinate males in the population were distinguished. Details of the technique were described previously [4]. It was shown that the distribution of the male mice among hierarchic classes depends on genotype. The highest percentage of dominant males was found in the PT line, the lowest in the CBA/Lac line [5]. The animals were killed by decapitation immediately after isolation (control), and 1, 7, and 48 h after group formation. The hypothalamus, hippocampus, and medulla, including the pons, were isolated in the mice in the cold. Activity of tyrosine hydroxylase (TH) was determined by measuring the rate of hydroxylation of tyrosine [13], in the modification in [1]. The protein concentration was recorded by Lowry's method [10]. Levels of mRNA of TH were analyzed by the dot hybridization method on nitrocellulose. Total RNA was isolated by phenolic extraction with guanidine thiocyanide [8]. Plasmid pBS (+/—) with the genome of rat TH, containing 1151 nucleotides inserted into its cDNA (D. M. Chikaraishi, Tufts Medical Center, Boston, Ma.), was used as the probe. The plasmid was labeled with ^{32}P by nick-translation up to specific activity of $4.4 \cdot 10^8$ counts/μg DNA/min. cDNA of TH in the composition of the RNA preparations was determined quantitatively by scanning autoradiographs on a DP-1 densitometer.

EXPERIMENTAL RESULTS

The formation of the hierarchic structure of the population is connected with aggressive confrontations between males, the intensity of which is greatest during the first few hours of its formation [2]. Later, aggressiveness declines

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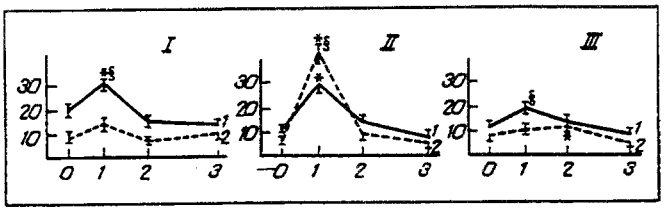

Fig. 1. Dynamics of brain TH activity during formation of hierarchic population structure. I) Hypothalamus, II) hippocampus, III) medulla. Ordinate, TH activity in nmoles dopa/min/mg protein; abscissa, time of existence of animals in population: O) control, 1) 1 h, 2) 7 h, 3) 48 h. Curves: 1) PT, 2) CBA/Lac. *p < 0 05 Compared with control. §) p < 0.05. Compared with CBA/Lac.

sharply, and after 2 days a hierarchy is established, the process being regarded as the etiologic mechanism of maintenance of stability of isolated communities. In the phase of aggressive confrontations, central catecholaminergic systems were activated (Fig. 1). In CBA/Lac mice, occupying a subordinate position in the population hierarchy [5], TH activity as early RS 1 h after group formation was increased by 30%, and was restored after 7 h. In PT males, occupying a dominant position, TH activity was increased by 60% after 1 h and after 7 h it was comparable with activity of the enzyme in the control animals. The increase in TH activity in the hypothalamus in PT males was significantly higher than that in CBA/Lac.

In the hippocampus, TH activity in mice of both lines was increased as early as 1 h after group formation: by 7 times in CBA/Lac and by 3 times in PT, whereas it was completely normalized after 7 h.

In the medulla of male CBA/Lac mice TH activity was unchanged 1 h after the beginning of formation of dominant-subordinate relationships. Increased TH activity was observed in them only toward 7 h, by 30% compared with the control animals, and these values returned to their initial levels by the 2nd day of their stay in the group. In PT males increased TH activity took place, after 1 h by 100%, but after 7 h its activity was back to the control level. Thus in male PT mice, genetically predisposed to dominance [5], and dominant in the population, intensification of catecholamine biosynthesis in the medulla appeared sooner and was more marked.

The TH mRNA level 1 h after the beginning of social stress was increased only in PT males, and not in all the brain structures studied, moreover (Fig. 2). In the medulla, for instance, the number of mRNA rose by 200%, in the hypothalamus it rose by 50%, but in the hippocampus no change was found in expression of the TH gene. In CBA/Lac males in the acute period of formation of dominant-subordinate relations, no changes in mRNA content were found in any of the parts of the brain studied.

During social stress connected with the formation of a hierarchy in the population, intensification of biosynthesis thus takes place in the catecholamine systems of the brain; activity of biosynthesis, moreover, is more marked in PT males, dominant in the population. In the region of the medulla, where the bodies of catecholaminergic neurons, which in the opinion of many authorities are the center of modulation of behavioral and endocrine reactions to stress [3, 7, 11], are concentrated, not only is more marked activation of catecholamine biosynthesis observed in dominant animals, but such activation takes place more rapidly, due to strengthening of activity of TH, the key enzyme of their synthesis.

Aggressive interactions between males during the acute period of social stress have an effect on the intensity of template activity in neurons, and give rise to potentiation of the transcription of TH mRNA. Intensification of expression of the TH gene is observed only in PT males in the region of the hypothalamus and medulla. In these mice, genetically predisposed to dominance, TH activity and functional activity of the TH gene exceed these parameters in CBA/Lac males. In the hippocampus, however, changes in activity of the enzyme are unconnected with a change in expression of the TH gene in this structure They are evidently mediated by changes taking place actually at the post-transcription level of TH regulation. The causes of the increased TH activity in the hippocampus may be different: intensification of phosphorylation of the enzyme [9], an increase in the content of the coenzyme of the hydroxylation reaction [12], intensification of transport of TH into the hippocampus from the regions of its biosynthesis or, finally, a combination of these processes [7].

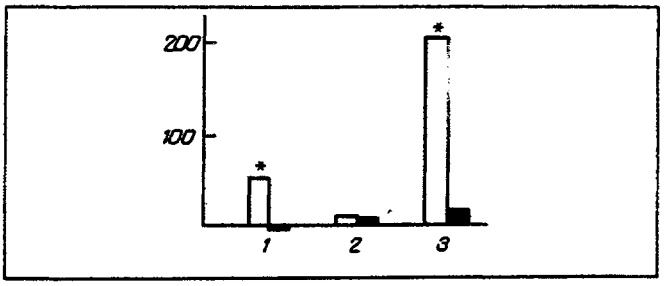

Fig. 2. TH mRNA level (in % of control) 1 h after formation of micropopulation. 1) Hypothalamus, 2) hippocampus, 3) medulla, unshaded columns – PT males, black columns — CBA/Lac. *p < 0.001 Compared with control of the same line.

It is a noteworthy fact that the increase in activity of TH and of its mRNA is unequal in the medulla and hypothalamus of PT males, In the medulla, for instance, the increase in TH mRNA is greater than the increase in activity of TH itself, whereas in the hypothalamus the opposite is true. In the hippocampus, despite an almost threefold increase in TH activity, no changes were found in expression of the TH gene. These results are evidently proof that part of the newly synthesized TH is transported from the locus coeruleus along axons into the hypothalanus and hippocampus. In addition, our results suggest that regulation of the functional activity of the TH gene depends on the brain structure in which it works.

In social stress associated with the formation of a hierarchy in the population, catecholamine biosynthesis in the mouse brain is thus increased. This increase takes place through an increase in activity of tyrosine hydroxylase, the key enzyme of catecholamine biosynthesis, and enhanced expression of its gene, Meanwhile these processes depend on the animal's genotype and on its position in the population structure.

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