EXPERIMENTAL BIOLOGY

Concentration of Monoaminergic Neurotransmitters and Their Major Metabolites in the Hippocamp and Brain Stem in Mice after Administration of Adrenocorticotropic Hormone ($ACTH_{4-10}$)

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$ACTH_{4-10}$ increases the concentration of monoaminergic neurotransmitters and the number of their metabolites in the brain of CBA and 101/HY mice. Different reactions to the peptide were revealed in both strains: the alterations were found either in brain stem (CBA strain) or in the hippocamp (101/HY strain).

Key Words: *$ACTH_{4-10}$; monoamines; early influences; brain development; strain mice*

There are many experimental models in which so-called early influences are used, i.e., administration of various biologically active agents during the early postnatal ontogenesis to modify the development of the central nervous system. There is evidence that some effects on the brain during neuronal differentiation may change the fate of mature neuron. This is indirectly indicated by shifts in cerebral neurochemical parameters in mature animals subjected to pre- and neonatal injections of various biologically active substances, such as testosterone, insulin, ACTH or its fragments, 6-hydroxydopamine, etc. [2,7-10]. Our aim was to study the effect of neonatal administration of $ACTH_{4-10}$ on the development of the monoaminergic brain systems in mice of two different inbred strains. Biochemical characteristics of cerebral monoaminergic systems were compared in intact 101/HY and CBA/Lac/Sto mice, which have behavioral differences [1]. The effect of neonatal injections of $ACTH_{4-10}$ on the state of these cerebral system in mature animals was evaluated in each strain.

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MATERIALS AND METHODS

The synthetic peptide $ACTH_{4-10}$ (Sigma) was dissolved in distilled water and injected subcutaneously in a dose of 5 μg/kg body weight from postnatal day 2 until day 7. The mice belonged to 101/HY (n=8) and CBA/Lac/Sto (n=12) inbred strains. The mice of each strain was subdivided into 3 groups: an experimental group and two control groups. The mice of the experimental group were injected with $ACTH_{4-10}$, the mice of the first control group of the same age were injected with distilled water, and the mice of the second control group were subjected to handling only.

The eight-month-old mice were decapitated, the brain was extracted in the cold and immediately placed into liquid nitrogen. High-pressure liquid

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chromatography was used to measure the concentrations of norepinephrine, dopamine (DA), 3,4-dioxyphenylacetic acid (DOPAA), serotonin (5-hydroxytriptamine, 5-HT), and 5-oxyindolylacetic acid (5-OIAA) in the hippocamp and brain stem. The data were statistically analyzed using nonparametric analysis (Statistica software). The intensity of dopamine (DOPAA/DA) and serotonin (5-OIAA/5-HT) metabolism, and the DA/5-HT ratio were calculated for the hippocamp and brain stem.

The data were used to calculate the mean concentration of each examined neurotransmitter and metabolite for every group of mice. The data of both control groups were united, because there were no significant differences between them. The differences between males and females were insignificant in all cases.

RESULTS

Comparison of control mice of both strains revealed that the contents of DOPAA and DA, as well as the DA/5-HT ratio were higher by about 2-fold in the hippocamp of 101/HY mice than of CBA mice (respectively, $p<0.02$ and $p<0.05$). By contrast, the contents of norepinephrine, 5-HT, and 5-OIAA did not significantly differ in these mice. The contents of norepinephrine and 5-HT were approximately 2-fold higher, and the 5-HT ($p<0.01$, Fig. 1) metabolism was enhanced in the brain stem of 101/HY mice in comparison with CBA mice. The contents of other neurotransmitters were the same in the brain stem in both strains.

Statistically significant difference in comparison with the control group was revealed in hippocampal monoamines (Fig. 1, *a*) of 101/HY mice which were injected with $ACTH_{4-10}$ in the neonatal period: the norepinephrine content and the DA/5-HT ratio increased about 2-fold ($p<0.01$), while the level of DOPAA decreased ($p<0.02$). No changes were revealed in the brain stem of these mice.

The effect of neonatal $ACTH_{4-10}$ on the monoamine content in the brain stem was observed in CBA mice but not in 101/HY mice (Fig. 1, *b*): the levels of norepinephrine ($p<0.01$), DOPAA ($p<0.05$), 5-OIAA ($p<0.01$), and 5-HT ($p<0.01$) increased, and 5-HT metabolism was intensified. No changes were revealed in the hippocamp.

Thus, the strongest effect of neonatal $ACTH_{4-10}$ was revealed in the hippocamp of 101/HY mice and in the brain stem of CBA mice. The differences between the strains in the reaction to the peptide are probably related to the different contents of monoamines in the control mice. The common feature for both strains was an increased content of the transmitters and/or their metabolites after neonatal $ACTH_{4-10}$. The local variations of the monoamines and the stability of their content may be related to the initial state of these systems in the mice of both strains. For example, the lower content of the brain stem monoamines in control CBA mice could underlie the significant increase of their level by $ACTH_{4-10}$. The changes were not observed in 101/HY mice probably because the control mice had relatively high initial levels of the brain- stem monoamines. However, this reasoning cannot explain the differences in the hippocampal monoaminergic systems: although the level of norepinephrine was similar in control mice, its content was significantly increased by neonatal peptide in 101/HY mice, while in CBA mice it was not changed.

$ACTH_{4-10}$ is known to modify the cerebral monoaminergic systems. In mature rats this peptide induced the accumulation of catecholamines, enhan-

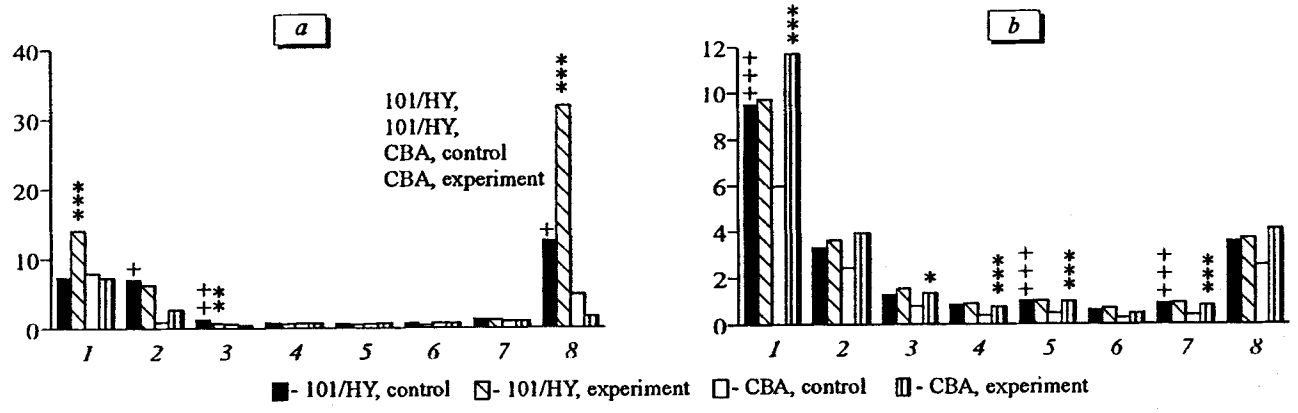

Fig. 1. Changes in the concentration of monoaminergic neurotransmitters and their metabolites in (*a*) hippocamp and (*b*) brain stem of adult CBA/Lac/Sto and 101/HY mice caused by neonatal administration of $ACTH_{4-10}$. *1*) norepinephrine; *2*) dopamine; *3*) 3,4-dioxyphenylacetic acid; *4*) 5-oxyindolylacetic acid; *5*) serotonin; *6*) dopamine metabolism; *7*) serotonin metabolism; *8*) dopamine/serotonin ratio. $^{*}p<0.05$, $^{**}p<0.02$, $^{***}p<0.01$ in comparison with the control group; $^{*}p<0.05$, $^{**}p<0.02$, $^{***}p<0.01$ in comparison with CBA mice.

ced their synthesis in the hypothalamus, thalamus, midbrain, and myelencephalon [10], and accelerated the restoration of their level after reserpine [3]. The peptide stimulated the metabolism and increased the content of norepinephrine in the macula cerulea [5]. The same effect was revealed in adrenalectomized rats [9]. Enhancement of norepinephrine metabolism was found when $ACTH_{4-10}$ was administered intraventricularly [4]. In all these cases either random-bred animals or the animals of a single strain were used.

Our data show that neonatal injections of $ACTH_{4-10}$ fragments modify cerebral monoaminergic systems in pubertal CBA and 101/HY mice. It should be stressed that this peptide did not modify the vitality of the mice, although it has a long-term effect. This is consistent by the data on increased activity of tyrosine hydroxylase in the integral brain after neonatal injection of $ACTH_{4-10}$ [7].

The differences between the mice in the reaction to pharmacological preparations and chemical agents have been known for a long time. For example, chronic administration of alcohol to mice of various strains induced various alterations in the state of cerebral monoaminergic systems [6]. Our data indicate that modification of these systems by neonatal injections of $ACTH_{4-10}$ are genetically determined.

Our results indicate that $ACTH_{4-10}$ affects the brain development. The method of the early influences of biologically active substances could be used for further study of the specific cellular and molecular mechanisms of early chemical intervention. This approach can be used to study the effects not only of $ACTH_{4-10}$, but also of other peptides or steroids that regulate neurogenesis.

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