Role of Reversible Phosphorylation in Genetically Determined Polymorphism for Cerebral Tryptophan Hydroxylase

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Experiments carried out to study the *in vitro* effects of phosphorylation and dephosphorylation on the activity of the key enzyme of serotonin biosynthesis, tryptophan hydroxylase, from the brain of C57Bl and BALB mice revealed a higher level of endogenous phosphorylation of the enzyme in the brain of BALB mice. The higher maximal activity of the enzyme derived from the brain of C57Bl mice appears to reflect increased expression of tryptophan hydroxylase in the brain of animals of this strain in comparison with BALB. It is possible that interstrain differences in cerebral tryptophan hydroxylase activity are caused mainly by two genetically determined factors: enzyme expression and its reversible phosphorylation.

Key Words: *tryptophan hydroxylase; phosphorylation; inbred mice*

Tryptophan hydroxylase (TPH) catalyzes tryptophan hydroxylation, the first and key stage of serotonin biosynthesis. Previously hereditarily determined polymorphism for TPH activity was revealed in the mouse brain and differences in the enzyme activity between C57Bl and BALB mice were shown to be determined by one locus [3]. TPH activity may rise as a result of an increase in the number of the enzyme molecules or due to posttranslation modification of already existing molecules. At present only one mechanism of posttranslation increase of TPH activity is known: reversible phosphorylation of the enzyme catalyzed by Ca, calmodulin-dependent proteinkinase, and Ca^{2+} [4]. Depolarization of serotoninergic neurons by electric current [5] or KCl [6] activates TPH phosphorylation.

This research was aimed at ascertaining the contribution of reversible phosphorylation to the determination of differences in TPH activity in mice.

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MATERIALS AND METHODS

Experiments were carried out with adult male C57Bl/6 and BALB/c mice with contrasting TPH activity [3]. Animals aged 3 months weighing 20 to 25 g were kept under standard conditions. Mice were decapitated and the brain stem (including the mesencephalon, pons, and medulla oblongata containing serotonin neuron bodies) was isolated in the cold, frozen with liquid nitrogen, and kept at -70°C. The brain stem was homogenized in 5 volumes of cold 0.05 M Tris-acetate buffer, pH 7.6, with 1 mM dithiothreitol and centrifuged for 30 min at 18,000 g (4°C). TPH activity was assessed in the supernatant by the fluorometric micromethod [2]. For a study of phosphorylation, the supernatant from one animal was divided into 3 portions and the enzyme activity was measured in the intact preparation (first portion), after phosphorylation with endogenous proteinkinase (second portion), and after dephosphorylation (third portion). TPH phosphorylation was carried out by adding adenosine triphosphate (ATP), $MgCl_2$, and $CaCl_2$ to the incubation medium to attain concentrations of 0.5, 5.0, and 0.1 mM, re-

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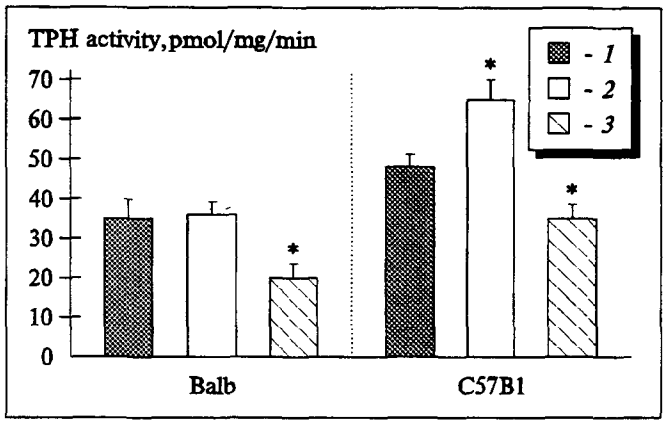

Fig. 1. Effect of *in vitro* incubation under phosphorylating conditions or with alkaline phosphatase on the activity of TPH from the brain stem of C57Bl and BALB mice. The enzyme activity was assessed after incubation of supernatant with 0.8 mM L-tryptophan and 0.5 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine (37°C, 15 min). *1*) native enzyme activity; *2*) TPH activity after incubation under phosphorylating conditions; *3*) TPH activity after incubation with alkaline phosphatase. The data of 6 measurements are presented. Asterisk indicates $p<0.001$ *vs.* the activity of the native enzyme.

spectively. Dephosphorylation was carried out by preincubating the supernatant before adding the substrate and cofactor for 3 min at 37°C with 0.1 U alkaline phosphatase (Boehringer) [5]. The V_{max} and K_m values of the enzyme were assessed by incubating the supernatant with different concentrations of L-tryptophan and calculated by the method of least squares [1]. TPH activity was expressed in pmoles of 5-hydroxytryptophan forming in 1 min converted to 1 mg of protein after Lowry. Statistical assessment was carried out using MANOVA for dependent samplings (CSS IBM/PC software package) and the Student *t* test.

RESULTS

TPH from the brain of C57Bl mice was characterized by high V_{max} values (55.1±3.3 pmol/mg/min) in comparison with the enzyme from the brain of BALB animals (33.4±3.3 pmol/mg/min, $p<0.001$). The K_m values of the enzyme did not appreciably differ in C57Bl and BALB mice (0.15±0.03 and 0.09±0.02 mM, respectively, $p>0.05$).

Comparison of changes in the activity of TPH from the brain of mice of these two strains for incubation of the enzyme under phosphorylating conditions (ATP, Mg^{2+} and Ca^{2+}) or with alkaline phosphatase *in vitro* revealed an appreciable influence of the genotype, phosphorylation, and interaction thereof on TPH activity (Table 1). Incubation of TPH from the brain of C57Bl mice under phosphorylating conditions boosted the enzyme activity ($p<0.01$). Preincubation with alkaline phosphatase markedly reduced the activity of TPH from the brain of C57Bl mice ($p<0.01$, Fig. 1). The capacity of TPH to phosphorylate and dephosphorylate *in vitro* indicates incomplete phosphorylation of this enzyme in the brain of C57Bl mice. At the same time, incubation of TPH from the brain of BALB mice in the presence of ATP, Mg^{2+}, and Ca^{2+} did not change the enzyme activity. Dephosphorylation reduced the activity of TPH from the brain of BALB mice ($p<0.01$, Fig. 1). Hence, TPH is completely phosphorylated in the brain of healthy BALB mice and cannot be additionally phosphorylated either *in vitro* or *in vivo*. The higher activity of the enzyme in the brain of C57Bl mice cannot be explained by posttranslation modification of TPH and appears to be caused by an increased concentration of the enzyme molecules. Increased expression of TPH in the brain of C57Bl mice is possible. Hence, hereditary polymorphism for TPH activity in the brain is determined by the expression of the enzyme and the process of its reversible phosphorylation. It is not known, however, whether these processes are regulated by one and the same or by different genetic factors.

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TABLE 1. Analysis of the Effect of Genotype and *in Vitro* Phosphorylation on the Activity of TPH in Mouse Brain

Factor	Degree of freedom	Mean square	Fisher test
Genotype	1	3165.9	90.1
Random deviation for genotype	20	35.2	–
Phosphorylation	2	1529.5	69.8
Genotype×phosphorylation	2	313.1	14.3
Random deviation	20	21.9	–

Note. For all values: $p<0.001$.

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Differential Repair Activity of Human Chromosomes

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The frequency of radioactive label (^{3}H-thymidine) incorporation in human lymphocyte chromosomes in the course of repair (extra) synthesis of DNA was assessed. Chromosomes 7, 12, and 21 were found to have a lower, and chromosome 22 a higher repair activity than was expected when a uniform incorporation of the label along the genome was hypothesized. Segments were detected that incorporated an increased number of labels in the course of extra DNA synthesis.

Key Words: *repair; human chromosomes; chromosome segments*

Differences in chromosome behavior in the course of replication, spiralization, and disjunction during cell division have been thoroughly studied [1,2]. There are reports of the differentiated activity of chromosomes in genetic processes related to the functioning of individual genes or to their interaction, which manifests itself in an increased frequency of sister chromatid exchanges and breaks at certain sites of chromosomes under the effect of mutagenic factors and substances "provoking" such phenomena [3,5,7]. As such data are accumulated, a picture of the complex behavior of chromosomes in the life cycle of the cell unfolds, observable by light microscopy using various methods. This picture is, however, incomplete because insufficient information is available about chromosome behavior in the course of extra DNA synthesis associated with repair of genome injuries. Such information would help assess the status of repair processes in the cell and its changes under the influence of both endogenous and exogenous factors.

This research was aimed at detecting differences in the activity of chromosomes or their individual sites in the course of repair DNA synthesis in the absence of mutagenic factors.

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MATERIALS AND METHODS

Whole blood of a healthy 30-year-old woman was used in the experiments. Newly isolated blood cells were incubated in a thermostat at 37°C in nutrient medium with ^{3}H-thymidine (final activity 20 μCi/ml) for 3 h. After incubation the cells were washed free of residual label and cultured in nutrient medium with phytohemagglutinin (PHA, PanEko, Moscow). After cell fixation and preparation of metaphase chromosomes, these preparations were coated with photosensitive emulsion and exposed in the dark at 4 C. After development of radioautographs the chromosomes were stained to detect G+ and G- segments. Chromosome identification and label scintillation and localization were carried out using microphotography and a map of the haploid set of chromosomes with 330 segments [4].

RESULTS

Microphotographs of 11 metaphase plates with a complete set of chromosomes were analyzed. A total of 890 labels were localized in chromosome

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