ROLE OF PHOSPHORYLATION IN ACTIVATION OF BRAIN TRYPTOPHAN HYDROXYLASE DURING HYPOTHERMIA I.

I. P. Voronova and A. V. Kulikov

UDC 612.82.015.06:612.592

KEY WORDS: tryptophan hydroxylase; phosphorylation; hypothermia

It was shown previously [1] that the increase in concentrations of serotonin and 5-hydroxyindoleacetic acid [4-6] and the intensity of mediator metabolism [10] observed in the rat brain during hypothermia can be explained by activation of the key enzyme of its biosynthesis, namely tryptophan hydroxylase (TPH, EC 1.14.16.4) [1]. However, the mechanism of this activation has not been explained. Activity of the enzyme may increase as a result either of synthesis of its molecules or of posttranslation modification of enzyme molecules already synthesized. It has been shown on brain slices that TPH is activated mainly through reversible phosphorylation of the enzyme by Ca^{2+}-calmodulin-dependent protein kinase [7]. There have been only sporadic reports [3, 9] that phosphorylation may be involved in TPH activation in the animal brain in response to external influences. Since activation of TPH in rats during cooling to a state of hypothermia has been observed quite quickly [1], it can be tentatively suggested that it is due to phosphorylation of the enzyme. The aim of this investigation was to study the role of phosphorylation in TPH activation in rats during hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats aged 2 months, weighing 200 g, and kept under standard conditions. In order to develop hypothermia, animals with temperature transducers fixed to them were placed in conical cages, restricting their freedom of movement, and they were cooled in water with ice. The body temperature was measured in the rectum at a depth of 6 cm by transducers based on MT-54M thermistors. Parameters were recorded by means of Shch4313 universal measuring instruments. Under the experimental conditions, the body temperature was lowered to 25°C in 7-10 min. The animals were then decapitated. Control rats, 10 min before decapitation, also were placed in conical cages but were not cooled. To determine TPH activity the midbrain, where the bodies of serotoninergic neurons are concentrated, and the striatum, where their endings are located, were isolated in the cold. The separate parts were homogenized in 5 volumes of cold 0.05 M Tris-acetate buffer, pH 7.5, containing 10^{-3} M dithiothreitol, and centrifuged for 30 min at 18,000*g* (4°C). TPH activity was determined in the supernatant by a fluorometric method [2]. To study phosphorylation, the supernatant obtained from one rat was divided into three parts: in the first part activity of the intact enzyme was determined, in the second part — after phosphorylation by endogenous protein kinase, and in the third part — after dephosphorylation. TPH was phosphorylated by addition of ATP, $MgCl_2$, and $CaCl_2$ to the incubation medium up to final concentrations of $5 \cdot 10^{-4}$ M, $5 \cdot 10^{-3}$ M, and 10^{-4} M respectively. Dephosphorylation was carried out by incubating the supernatant before addition of the substrate for 3 min at 37°C with 0.1 U of alkaline phosphatase ("Biolar," USSR) [8]. Since phosphorylation first of all increases affinity of the enzyme for the substrate, TPH activity was determined in the presence of a low concentration (10^{-4} M) of tryptophan. Activity of the enzyme was expressed in picomoles 5-hydroxytryptophan formed per minute, per milligram protein, measured by Lowry's method. The results were subjected to statistical analysis by the t test.

Laboratory of Phenogenetics of Behavior, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 56-57, July, 1991. Original article submitted August 28, 1990.

 0007-4888/91/0007-0972$12.50

TABLE 1. Effect of Phosphorylation and Dephosphorylation of TPH on Its Activity in the Striatum and Midbrain of Control Rats and Rats Cooled to a Body Temperature of 25°C

Part of brain	State of animal	Initial activity	TPH (pmoles/mg/min)	
			phosphorylation	dephosphorylation
Striatum	Control	6,0±1,0	9,9±1,0*	3,9±0,9*
	Hypothermia	8,9±0,6^{+}	7,3±0,7	3,4±0,4*
Midbrain	Control	26,9±1,6	53,7±3,9**	18,8±1,9*
	Hypothermia	36,3±1,7^{++}	59,8±2,9**	22,0±1,7*

Legend. TPH activity was measured in the presence of tryptophan 10^{-4} M and the cofactor 6,7-dimethyltetrahydropteridine $5 \cdot 10^{-4}$ M. Phosphorylation by endogenous protein kinase in the presence of $5 \cdot 10^{-4}$ M ATP, $5 \cdot 10^{-3}$ M $MgCl_2$, and 10^{-4} M $CaCl_2$. Dephosphorylation was carried out by preincubating the samples in the presence of 0.1 U alklaline phosphatase. *$p < 0.05$, **$p < 0.01$ compared with initial activity (with the aid of the paired t test); $^{+}p < 0.05$, $^{++}p < 0.01$ compared with control. Each series included at least nine animals.

EXPERIMENTAL RESULTS

TPH activity measured in the presence of a tryptophan concentration close to K_M (10^{-4} M) was higher in the striatum and midbrain of the hypothermic animals than in the controls (Table 1). Phosphorylation of the enzyme from the striatum by endogenous protein kinase increased TPH activity only in the control animals up to the level of activity of the intact enzyme found in the hypothermic animals. Meanwhile, phosphorylation did not significantly change TPH activity from the striatum of the cooled rats. Dephosphorylation lowered activity of the enzyme from the striatum of both groups of animals to the same level. However, under these circumstances activity of the enzyme in the control animals fell by 50% but in the hypothermic animals it fell by 2.5 times compared with activity of the intact enzyme (Table 1).

In the midbrain phosphorylation significantly increased activity of the enzyme in animals of both groups to the same level. Dephosphorylation led to reduction of activity of the enzyme compared with activity of intact TPH in both control and hypothermic rats; the decrease in enzyme activity, moreover, was more marked in the hypothermic rats (Table 1).

It can be concluded from these results that the enzyme was not completely phosphorylated in the brain structures tested in the control animals. Cooling the rats led to an increased degree of phosphorylation of TPH, and in the striatum, the enzyme was completely phosphorylated, so that no further phosphorylation was possible in vitro. In the midbrain, hypothermia did not induce complete phosphorylation of the enzyme, and its activity could be increased more by phosphorylation in vitro. However, short-term (not more than 10 min) hypothermia does not evidently lead to the synthesis of new TPH molecules, for both the completely phosphorylated and the completely dephosphorylated enzyme has the same activity in both control and hypothermic rats.

Thus during short-term hypothermia, in serotonin endings in the striatum and in the bodies of serotonin neurons in the midbrain, a rapid mechanism is activated for increasing TPH activity, namely reversible phosphorylation of the enzyme. Phosphorylation of TPH can explain the sharp increase in activity of the enzyme in the brain which was found during hypothermia [1]. Recently the present writers demonstrated the important role of phosphorylation of TPH in the development of catalepsy in rats [3]. Another group of workers [9] found intensification of TPH phosphorylation in the

brain of rats exposed to the action of a strong acoustic stimulus. Phosphorylation of TPH is evidently a common mechanism of rapidly increasing serotonin biosynthesis in the brain in response to external stimuli.

LITERATURE CITED

1. I. P. Voronova and E. Yu. Zhanaeva, The Striatal System and Behavior under Normal and Pathological Conditions [in Russian], Leningrad (1988), p. 21.
2. A. V. Kulikov, I. P. Voronova, and E. Yu. Zhanaeva, Vopr. Med. Khim., No. 2, 120 (1988).
3. A. V. Kulikov, E. Yu. Kozlachkova, L. E. Pirushkina, and N. K. Popova, Neirokhimiya (1991).
4. G. F. Molodtsova, The Most Important Theoretical and Practical Problems in Thermoregulation [in Russian], Novosibirsk (1982), p. 149.
5. É. Z. Emirbekov, R. A. Abdullaev, and I. I. Ibragimov, Ukr. Biokhim. Zh., **52**, 418 (1980).
6. É. Z. Emirbekov, I. I. Ibragimov, and R. A. Abdullaev, Ukr. Biokhim. Zh., **50**, 296 (1978).
7. M. C. Boadle-Biber, Biology of Serotoninergic Transmission, ed. by N. N. Osborn, New York (1982), pp. 63-94.
8. M. C. Boadle-Biber, J. N. Johannessen, N. Narasimhachari, and T. Phan, Neurochem. Int., **8**, 83 (1986).
9. M. C. Boadle-Biber, K. C. Corley, L. Graves, et al., Brain Res., **482**, 306 (1989).
10. C. Okuda, A. Saito, M. Miyazaki, and K. Kuriama, Pharmacol. Biochem. Behav., **24**, 79 (1986).