ACTIVATION OF TYROSINE HYDROXYLASE DURING ELECTRICAL STIMULATION OF ISOLATED NERVE ENDINGS OF THE RAT HYPOTHALAMUS

M. F. Mineeva-Vyalykh, V. K. Lutsenko, V. S. Kudrin, and O. P. Sakharova

UDC 612.826.4.015.11.014.424

The effect of electrical stimulation on synaptosomal membrane-bound tyrosine hydroxylase (TH) activity in the rat hypothalamus was investigated. Electrical stimulation intensified respiration and glycolysis, evidence of excitation of the synaptosomes. Against this background, activity of membrane-bound TH was increased. The value of K_m for tyrosine fell from 0.091 to 0.026 mM. Inhibition of the enzyme activity by the end product of cathecholamine biosynthesis (noradrenalin) was reduced. It is postulated that the effect of depolarization on the rate of catecholamine synthesis in nerve endings is effected through modification of TH.

KEY WORDS: hypothalamus; tyrosine hydroxylase; electrical stimulation; synaptosomes.

Excitation of brain structures is accompanied by activation of tyrosine hydroxylase (TH) [14]. In small doses, cocaine excites the CNS, but in vitro it activates TH of isolated nerve endings (synaptosomes) [3]. It was decided to obtain more direct information on the relationship between excitation of nerve endings and TH activity.

For this purpose the kinetic characteristics of TH were studied during depolarization of nerve endings. Depolarization of synaptosomes isolated from rat brain increases the liberation of catecholamines [5, 7] and the rate of their synthesis [13], and intensifies respiration and glycolysis by synaptosomes [6]. To characterize the native state or otherwise of the synaptosomes, their respiration and glycolysis were measured at rest and during stimulation. The synaptosomes were excited by electrical stimulation (ES) [2, 6].

EXPERIMENTAL METHOD

Synaptosomes were isolated from the hypothalamus of noninbred male albino rats weighing 180-200 g by the method of Gray and Whittaker [8]. The residue of synaptosomes was suspended in 0.1 M Krebs–Ringer phosphate buffer, pH 7.5 [6]. The suspension was transferred to the small containers of Warburg flasks in which concentric platinum wire (diameter 0.5 mm) electrodes were soldered. During the experiment these small containers were connected together electrically in series to the output of an ÉSU-1 stimulator. Two of them contained the synaptosomes for stimulation; the other two contained incubation medium. The medium was used to study the thermal and electrochemical effects of the electric current. Two similar pairs of small Warburg containers formed the control group. In all the experiments the strength of the current (mean continuous current 10 mA), the frequency of stimulation (50 pairs of square pulses of opposite polarity per second), and pulse amplitude (3-4 V) were applied to the container for 0.4 msec. Equality of amplitudes of opposite polarity was verified by a Tesla pulse voltmeter and the shape of the pulse was monitored on the screen of an S1-18 cathode-ray oscilloscope.

After the end of ES the contents of the small containers were centrifuged for 10 min at 25,000g to separate the synaptosomes from the incubation medium. The concentrations of pyruvic and lactic acids in the

Laboratory of Neurochemical Pharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR. Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 543-545, May, 1977. Original article submitted November 17, 1976.

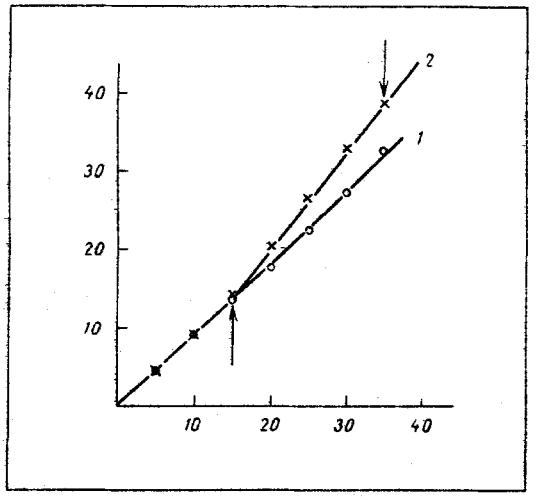

Fig. 1. Oxygen consumption of suspension of rat hypothalamic synaptosomes at rest (1) and during ES (2). Arrows mark beginning and end of ES. Abscissa, time (min); ordinate, oxygen consumption (in μmoles O_2/100 mg protein/h).

supernatant were determined by enzymic methods [1]. The synaptosomes were disintegrated osmotically by keeping them in hypotonic 0.001 M K-phosphate buffer, pH 7.0, containing 0.001 M $CaCl_2$. The remaining suspension was treated by the method of Kuczenski and Mandell [10] to obtain the synaptosomal membrane-bound TH. Activity of TH was measured at 26°C by a spectrophotometric method [4] and protein by Lowry's method [11]. To obtain synaptosomes, the brain tissue of 40 animals was used in each experiment. The results of five experiments are described below.

EXPERIMENTAL RESULTS

The rate of respiration of the synaptosomes remained unchanged during observation for 35 min, indicating that the synaptosomes were in the native state and that the incubation conditions were adequate. The presence of platinum electrodes had virtually no effect on the oxygen consumption. The absolute values of the oxygen consumption of the control samples agreed with data in the literature [6].

Electrical stimulation of the synaptosomes was accompanied by an increase in the rate of respiration (Fig. 1) and a marked increase in glycolysis: The pyruvate concentration rose by 50% (Table 1). The lactate concentration was increased by only 13%, probably on account of a decrease in lactate dehydrogenase activity. Thus, ES evoked a distinct metabolic response of the synaptosomes and these results coincided with results obtained by other workers using different methods of depolarization of the synaptosomes [6].

Incubation of the synaptosomes for 35 min in Krebs–Ringer medium at 37°C had a marked effect on the properties of the membrane-bound TH isolated from them, as was manifested in particular by a decrease in the affinity of the enzyme for the substrate. Under these conditions the value of K_m for the control samples ($K_m = 0.091 \times 10^{-3}$ M) was almost twice the value (0.045–0.055 mM) given in the literature [4, 10] for TH immediately after isolation from the brain. Electrical stimulation of the synaptosomes activated the membrane-bound enzyme considerably (Fig. 2). In all the experiments an increase in the initial reaction velocity was observed. Analysis of the curve showing the reaction velocity as a function of substrate concentration showed that the affinity of the enzyme for tyrosine was increased after ES. As Fig. 2 shows, the value of K_m was reduced by 71.4% compared with the control (0.026 mM). TH is known to be controlled by the end product of the chain of reactions of catecholamine synthesis, namely noradrenalin. Accordingly, experiments were carried out to study the effect of 0.8 mM noradrenalin on TH activity. In the control samples noradrenalin in this concentration inhibited the activity of the enzyme on average by 96%. Inhibition of TH by noradrenalin after ES of the synaptosomes was 73%, i.e., it was reduced by 23%. The study of the relationship between TH activity and the concentration of the coenzyme $DMPH_4$ showed a difference between the enzyme preparations from the stimulated and control synaptosomes. Special analysis is required to elucidate the character of these differences.

TABLE 1. Concentration (in μmoles/100 mg protein) of Lactic and Pyruvic Acids in Suspension of Rat Hypothalamic Synaptosomes at Rest and during ES (M ± m)

Metabolites	Incubation medium		Residue of synaptosomes	
	control	ES	control	ES
Pyruvate	2,05±0,52	2,83±0,54	1,01±0,22	1,51±0,22
Lactate	5,95±0,24	6,74±0,28	2,95±0,15	3,39±0,16

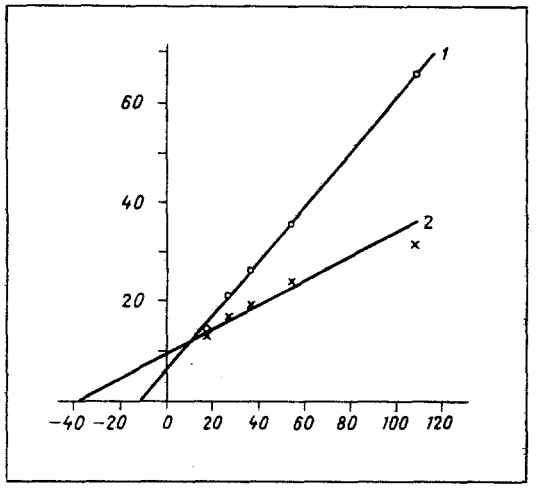

Fig. 2. Velocity of tyrosine hydroxylase reaction as a function of tyrosine concentration at rest (1) and during ES (2). Buffer: 0.1 M Tris-maleate, pH 6.15. Concentration of coenzyme 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) 0.185 mM. Abscissa, 1/[tyrosine] × 10^6 (in moles); ordinate, 1/reaction velocity (in mM^{-1} h/min/mg protein).

In a recently published paper by De Belleroche et al. [9] increased dopamine synthesis during ES of synaptosomes isolated from the corpus striatum of the sheep brain was described. The increase in dopamine synthesis was found only when labeled tyrosine and not 3,4-dihydroxyphenylalanine was used. Similar results were obtained with depolarization induced by veratridine [13].

The fact of modification of TH, manifested as a change in the kinetic properties of the enzyme and a decrease in its sensitivity to inhibition by the end product (noradrenalin), established by these experiments, suggests that the key factor in the increased rate of catecholamine biosynthesis during depolarization of adrenergic nerve endings is a change in the regulator properties of TH.

Modification of the enzyme may be due to Ca^{2+} ions entering the synaptosomes on depolarization [5]. Changes in the kinetic properties of the membrane-bound enzyme on depolarization induced by ES are similar to those observed for soluble TH in the presence of Ca^{2+} ions [12].

LITERATURE CITED

1. V. S. Asatiani, Enzymic Methods of Analysis [in Russian], Moscow (1969).
2. R. N. Glebov, N. M. Dmitriev, V. K. Lutsenko, et al., Dokl. Akad. Nauk SSSR, 215, 1247 (1974).
3. V. V. Zakusov and M. F. Mineeva-Vyalykh, Byull. Éksp. Biol. Med., No. 9, 1071 (1976).
4. M. F. Mineeva-Vyalykh, Vopr. Med. Khim., No. 2, 274 (1976).
5. M. P. Blaustein, J. Physiol. (London), 247, 617 (1975).
6. H. F. Bradford, G. W. Bennett, and A. J. Thomas, J. Neurochem., 21, 485 (1973).
7. C. W. Cotman, J. W. Haycock, and W. F. White, J. Physiol. (London), 254, 475 (1976).
8. E. G. Gray and V. P. Whittaker, J. Anat., 96, 79 (1962).
9. J. S. De Belleroche, H. F. Bradford, and D. G. Jones, J. Neurochem., 26, 561 (1976).
10. R. R. Kuczenski and A. J. Mandell, J. Biol. Chem., 247, 3114 (1972).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).

12. V. H. Morgenroth, M. C. Boadle-Biber, and R. H. Roth, Mol. Pharmacol., 11, 427 (1975).
13. R. L. Patrick, T. E. Snyder, and J. D. Barchas, Mol. Pharmacol., 11, 621 (1975).
14. D. S. Segal, J. Y. Sullivan, R. Kuczenski, et al., Science, 173, 897 (1971).

ROLE OF THE ADENYLATE CYCLASE SYSTEM IN INDUCTIVE ACETYLCHOLINESTERASE SYNTHESIS IN THE BRAIN

N. G. Aleksidze and M. V. Balavadze

UDC 612.82+577.15

The increase in acetylcholinesterase (ACE) activity in the rat brain after intraventricular injection of adrenalin and the dibutyryl analog of cyclic AMP was shown to be the result of inductive synthesis of the enzyme. Induction of ACE is manifested to a greater degree in the white matter of the subcortex than in the cortex. Blocking β-adrenergic receptors inhibits the stimulating action of adrenalin on ACE activity but does not alter the effect of cyclic AMP. Blocking of the α-adrenergic receptors, on the other hand, potentiates induction of synthesis of the enzyme. The effects of adrenalin and of dibutyryl-cyclic AMP are similar in direction and are mediated through β-adrenergic receptors. The increase in ACE activity after blocking of α receptors can be explained by the elimination of their inhibitory effect on β-adrenergic receptors.

KEY WORDS: acetylcholinesterase; rat brain; α- and β-adrenergic receptors; cyclic nucleotides; induction of enzyme synthesis.

Evidence of the stimulating effect of adrenalin and of cyclic AMP on acetylcholinesterase (ACE) activity of the animal brain has been published [1, 4, 5, 9], but the mechanism of the potentiation of activity of this enzyme has not yet been explained.

The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

The brain of growing albino rats weighing 80-100 g was used. The following doses of the various substances were tested (per 100 g body weight): noradrenalin or adrenalin 5, 10, and 20 μg, dibutyryl-cyclic AMP 5 μg, euspiran (isoprenaline) 2.5, 5, and 10 μg, puromycin 90 μg, anaprilin (propranolol), blocking β-adrenergic receptors, 6.5-12.5 μg, and atropine, a cholinolytic, 1.25-2.50 μg. The substances used were made up in physiological saline and injected intraventricularly, bilaterally, directly into the 3rd ventricle by means of a microsyringe. Activity of ACE was determined by Eliman's method [7] in the cortex and in the subcortical white matter of the frontal lobes of the rats' brain.

EXPERIMENTAL RESULTS

The experiments of series 1 showed an increase in ACE activity both in the cortex and in the subcortical white matter after an exposure of 1 h to adrenalin injected into the brain in doses of 5, 10, and 20 μg (Table 1). The increase in enzyme activity correlated with the dose of adrenalin injected. The ACE activity also increased gradually with time (Fig. 1), evidence of induction of synthesis of the enzyme.

Department of Biochemistry, Tbilisi University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 545-548, May, 1977. Original article submitted November 17, 1976.