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

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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.

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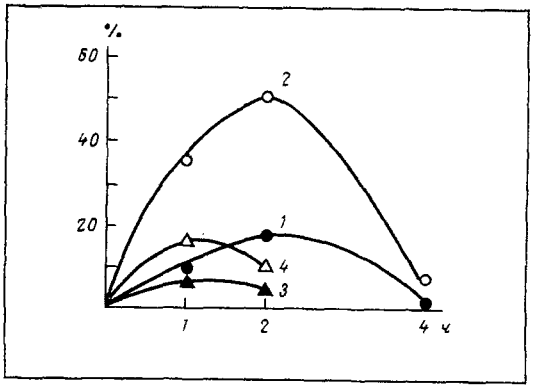

Fig. 1. Effect of intraventricular injection of adrenalin alone (5 μg) and adrenalin combined with the β adrenoblocker propranolol (6.5 μg) on ACE activity in cortex and subcortical white matter of frontal lobe of rat brain: 1, 3) cortical ACE activity after injection of adrenalin alone and adrenalin with propranolol; 2, 4) subcortical ACE activity after injection of adrenalin alone and adrenalin with propranolol. Abscissa, exposure (in h); ordinate, ACE activity (in % of control).

TABLE 1. Effect of Intraventricular Injection of Adrenalin, Puromycin, Euspiran, and Noradrenalin on ACE Activity in Cortex and Subcortical White Matter of Frontal Lobes of the Rat Brain (M ± m)

Experimental conditions	Dose, μg/100 g body weight	Time of exposure, h	Cortex		Subcortex	
			ACE	% of control	ACE	% of control
Control (15)	—	—	3,13±0,05	100,0	3,80±0,06	100,0
Adrenalin (10)	5	1	3,37±0,08	107,7	5,15±0,15	135,5
(6)	10	1	3,52±0,07	112,5	5,37±0,11	141,3
(8)	20	1	3,87±0,08	123,6	5,17±0,16	136,1
Puromycin + adrenalin (8)	90	2	3,26±0,43*	104,2	3,75±0,24*	98.7
	20	1				
Euspiran (6)	2,5	2	3,13±0,06*	100,0	4,14±0,07	108,8
(6)	5	2	3,30±0,05*	105,4	4,85±0,05	127,6
(6)	10	2	3,33±0,09*	106,4	5,32±0,14	140,0
Noradrenalin (7)	5	1	3,09±0,05*	98,7	4,16±0,12	109,5
(8)	10	2	3,33±0,05*	106,4	4,12±0,09	108,4

Legend. Here and in Table 2: 1) enzyme activity expressed in 10^{-4} M acetylcholine/h/g wet weight of tissue; 2) number of experiments in parentheses; 3) values for which $P < 0.05$ compared with control marked by asterisk.

The stimulating effect of adrenalin disappeared completely after preliminary injection of puromycin, which inhibits protein synthesis, into the brain (exposure 2 h). This fact is fundamental evidence of induction of the synthesis of ACE molecules by adrenalin.

Euspiran, an analog of adrenalin, also stimulated brain ACE activity. Depending on the dose injected (2.5, 5, or 10 μg) the activity of the enzyme in the subcortical white matter after an exposure of 2 h rose by 8.6, 27.6, and 40%, respectively. Noradrenalin, in a dose of 5-10 μg, had a weak effect on the enzyme activity. It exhibited a stimulating action on ACE activity only in the subcortical white matter: The activity of the enzyme increased by 9.5% during an exposure of 2 h.

After intraventricular injection of the dibutyryl analog of cyclic AMP in a dose of 5 μg an increase in ACE activity also was observed, but chiefly in the subcortical white matter. In this case, after an exposure of 4 h, the activity of the enzyme was 60.8% higher than in the control. In the cortex, where the highest phosphodiesterase activity is found [6], ACE showed no significant change (Table 2). Puromycin completely inhibited the stimulating effect of dibutyryl-cyclic AMP, and also of adrenalin, on the brain ACE activity.

The experiments thus showed that cyclic AMP plays the role of secondary mediator in the stimulation of brain ACE. To verify this conclusion, the role of α- and β-adrenergic receptors in the inductive synthesis of ACE molecules was studied.

TABLE 2. Effect of Intraventricular Injection of Dibutyryl-Cyclic AMP, Puromycin, and Propranolol, Blocking β-Adrenergic Receptors, on ACE Activity in the Cortex and Subcortical White Matter of Frontal Lobe of the Rat Brain (M ± m)

Experimental conditions	Dose, μg/100 g body weight	Time of exposure, h	Cortex		Subcortex	
			ACE	% of control	ACE	% of control
Control	—	—	3,13±0,05	100,0	3,80±0,06	100,0
Dibutyryl-cyclic AMP	5	2	3,44±0,11	109,9	4,75±0,07	125,0
Dibutyryl-cyclic AMP + propranolol (4)	5 6,5	2	3,40±0,12	108,6	4,78±0,09	125,8
Dibutyryl-cyclic AMP + propranolol (5)	5 12,5	2	3,46±0,09	110,5	3,44±0,10	90,5
Dibutyryl-cyclic AMP	5	4	3,39±0,08*	108,3	6,11±0,18	160,8
Dibutyryl-cyclic AMP + propranolol (3)	5 6,5	4	3,39±0,19	107,9	6,09±0,07	160,3
Dibutyryl-cyclic AMP + propranolol (5)	5 12,5	4	3,25±0,11*	103,8	4,84±0,31	127,4
Dibutyryl-cyclic AMP (6)	5	24	3,21±0,12*	102,6	3,85±0,06*	101,3
Diburyryl-cyclic AMP + propranolol (5)	5 12,5	24	3,13±0,09*	100,0	3,76±0,04*	98,9
Propranolol (5)	12,5	2	2,79±0,10	89,1	2,50±0,21	65,8
Dibutyryl-cyclic AMP + propranolol (8)	90 5	4	3,07±0,05*	98,1	3,40±0,06	89,5

As Fig. 1 shows, after the simultaneous injection of adrenalin and propranolol, blocking β-adrenergic receptors, the positive action of adrenalin on ACE activity was completely abolished or, at least, appreciably reduced. Propranolol, alone in a dose of 0.5 μg or after dibutyryl-cyclic AMP (Table 2), had virtually no effect on brain ACE activity either in the cortex or in the subcortical white matter. With an increase in its dose to 12.5 μg, after an exposure of 2 h ACE activity fell by 34.2% (Table 2). The stimulating effect of dibutyryl-cyclic AMP in the subcortical white matter was reduced by about the same amount.

No changes in the ACE activity of brain homogenate could be found in the experiments in vitro after a change in the propranolol concentration in the incubation medium from 0.65 to 12.5 μg/ml. By contrast with the β adrenoblocker, propranolol, the α adrenoblocker phentolamine stimulated ACE activity in the subcortical white matter, which it increased by 18-68%. It must be concluded from these findings that blocking of the β receptor does not affect the biochemical conversions which lie at the basis of the inductive synthesis of ACE after injection of dibutyryl-cyclic AMP.

Considering that after injection of adrenalin into the animals the concentration of free acetylcholine (AC) in the brain is increased [8], the attempt was made to evaluate the role of AC in the inductive synthesis of ACE. To abolish the effects of AC, the cholinolytic drug atropine was injected into the brain. Atropine, in a dose of 1.25 μg (by bilateral intraventricular injection), either by itself or in the presence of dibutyryl-cyclic AMP, was found to have no significant effect on ACE activity in the cortex or subcortical white matter, but it reduced the stimulating action of adrenalin on the ACE activity in the cortical white matter on the average by 30% ($P < 0.05$). Atropine, in a dose of 2.25 μg, separately (without adrenalin) stimulated ACE activity after an exposure of 2 h by 32.9%. Hence, it must be concluded that the inducing action of adrenalin on ACE synthesis is due partly to AC. The increase in ACE activity produced by atropine can evidently be explained on the grounds that atropine, on the one hand, abolishes the inhibitory effect of ACE on adenylate cyclase, and on the other hand, stimulates adenylate cyclase [10-12].

According to data in the literature [2], atropine is a weak reversible inhibitor of ACE. This view conflicts with the results now obtained. However, it must be pointed out that the inhibitory property of atropine is manifested in a concentration of 250 μg/100 g body weight, which is about 50-100 times greater than the doses used under the conditions of the present experiments.

The great difference will be noted between the rate of the inductive action of adrenalin and of dibutyryl-cyclic AMP on ACE synthesis in the brain. Whereas this action of adrenalin is maximal after 2 h (Fig. 1), the greatest effect of dibutyryl-cyclic AMP is found after an exposure of 4 h (Table 2), when the action of adrenalin has completely disappeared. The explanation of this fact must be sought in the lower reactivity of exogenous dibutyryl-cyclic AMP. Adrenalin exerts its action through adrenergic receptors, with the use of endogenous cyclic AMP as secondary mediator. According to data in the literature, the inotropic and metabolic effects of adrenalin and cyclic AMP likewise occur at different times [3].

It is thus clear that the increase in brain ACE activity after intraventricular injection of adrenalin and dibutyryl-cyclic AMP is the result of inductive synthesis of the enzyme. The effects of adrenalin and of cyclic AMP are similar in direction and are mediated through β-adrenergic receptors. The potentiation of ACE activity after blocking of the α receptors can be explained by elimination of their inhibitory effect on the β-adrenergic receptors.

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A MULTICOMPONENT SYSTEM OF ESTRADIOL-BINDING PROTEINS IN RAT LIVER CYTOSOL AND ITS DEPENDENCE ON SEX STEROIDS

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The marked sex differences in the ratio between the hormonal capacity of estradiol-binding components with Stokes' radii (**a**) of 7.0 and 2.5 nm observed in sexually mature animals are somewhat reduced but do not disappear completely after gonadectomy. Prolonged administration of estradiol (50 μg, 8 days) to gonadectomized rats leads to depression of the estradiol-binding activity of all components of liver cytosol of females and males. Injection of testosterone propionate (2 mg, 8 days) into gonadectomized animals leads to selective stimulation of a special estrogen-binding protein with **a** = 2.5 nm, normally characteristic of males alone, in both males and females. It is postulated that sex differences in the system of estradiol-binding proteins of the rat liver cytosol are due to sexual differentiation of the system in the early stages of development, on the one hand, and to the active regulatory influence of androgens and estrogens in the late stages of development, on the other hand.

KEY WORDS: estrogens; estradiol-binding proteins; liver; sex steroids.

The liver of fishes, amphibians, and birds, under the influence of estrogens, can produce the egg proteins lipovitellin and phosphitin and can thus play a direct part in the reproductive process [7, 10]. In mammals, the effects of estrogens on the reproductive function mediated through the liver are not so clearly defined and they

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