ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE IN THE NERVOUS SYSTEM OF RABBITS PREDISPOSED TO CARDIOVASCULAR DISORDERS UNDER EMOTIONAL STRESS

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This investigation, which is a continuation of a study of the acetylcholine (ACh) system undertaken on animals with stable cardiovascular functions [3], is aimed at determining the degree of participation of components of the ACh system in the disturbance of self-regulation of functions in rabbits predisposed to cardiovascular disorders under the influence of an adverse emotiogenic stimulus.

EXPERIMENTAL METHOD

Experiments were carried out on male Chinchilla rabbits. Activity of acetylcholinesterase (AChE), choline acetyltransferase (CAT), and butyrylcholinesterase (BChE) and the concentration of water-soluble proteins were determined in structures of the CNS. Emotional stress was induced in immobilized animals by aperiodic stimulation of the ventromedial hypothalamic nuclei and by electrodermal stimulation. Square pulses (50 Hz, 1 msec, 5-10 V, 100-300 μA) were used. Stimulation was applied for 1-2 min with an interval of 30 sec-4 min. Rabbits whose blood pressure fell progressively during the 3rd hour of the experiment were chosen for investigation. The animals' brain was frozen and used to obtain slices 300 μ thick. The following formations were isolated: the medullary reticular formation (RF), the mesencephalic RF at the level of the superior colliculus (dorsal part), mesencephalic RF at the level of the superior colliculus (ventral part), caudal nucleus of the pons, perifornical region of the hypothalamus, and part of the neocortex (precentral region and medial part of the postcentral region) [1]. The following autonomic ganglia also were investigated: superior cervical and stellate sympathetic ganglia (SCG and SG respectively), and also the ganglion nodosum (GN) of the vagus nerve. AChE activity was determined by Ellman's method [5]. BChE activity was revealed by the use of butyrylthiocholine iodide as substrate. CAT activity was determined biochemically by the method in [8], using labeled acetyl-CoA. When investigating CAT, besides intact animals (control 1) rabbits of an additional group (control 2) with implanted electrodes, kept for 3 h under conditions of immobilization, also were used. The concentration of water-soluble proteins was determined by Lowry's method [7] in all samples of nerve tissue. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

AChE activity in structures of the CNS in rabbits predisposed to cardiovascular disorders under emotional stress was unchanged compared with control 1 (Table 1). Increased activity was found in ganglia of the autonomic nervous system under these conditions (in GN at $P < 0.01$, in SCG at $P < 0.1$). BChE activity was increased in the CNS structures investigated. The greatest changes in BChE level occurred in the cerebral cortex, GN, medullary RF, and caudal nucleus of the pons.

CAT activity was determined in the perifornical region of the brain of animals predisposed to cardiovascular disorders under emotional stress, and also in rabbits with stable

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TABLE 1. AChE and BChE Activity and Concentration of Water-Soluble Proteins in CNS Structures of Rabbits Predisposed to Cardiovascular Disorders under Emotional Stress (M ± m)

Structure	AChE, millimoles/min/mg protein		BChE, millimoles/min/mg/ protein		Protein, mg/mg tissue	
	control 1	experiment	control 1	experiment	control 1	experiment
Medullary RF	0,34±0,11 (7)	0,33±0,04 (7)	0,06±0,01 (7)	0,09±0,02*** (6)	0,04±0,01 (6)	0,03±0,00 (7)
Mesencephalic RF, dorsal part	0,31±0,10 (6)	0,35±0,06 (6)	0,07±0,02 (6)	0,14±0,06 (6)	0,03±0,01 (6)	0,03±0,01 (7)
Mesencephalic RF, ventral part	0,3±0,12 (5)	0,31±0,03 (6)	0,08±0,02 (7)	0,11±0,02 (6)	0,04±0,01 (5)	0,03±0,01 (7)
Caudal nucleus of pons	0,3±0,11 (7)	0,28±0,04 (7)	0,05±0,01 (6)	0,08±0,01*** (6)	0,03±0,01 (5)	0,03±0,00 (7)
Perifornical region	0,21±0,06 (4)	0,27±0,02 (6)	0,03±0,01 (4)	0,04±0,01 (5)	0,05±0,02 (4)	0,04±0,00 (6)
Cortex (precentral and medial part of postcentral region)	0,11±0,03 (7)	0,60±0,38 (6)	0,02±0,00 (7)	0,12±0,05** (7)	0,02±0,00 (5)	0,03±0,01 (9)
SCG	0,91±0,19 (7)	1,80±0,32*** (8)	0,97±0,24 (9)	1,73±0,42 (9)	0,07±0,00 (9)	0,05±0,01** (10)
SG	1,07±0,31 (8)	1,87±0,32 (9)	0,73±0,21 (8)	1,17±0,39 (9)	0,08±0,02 (8)	0,06±0,01 (10)
GN	0,63±0,12 (9)	1,21±0,14* (8)	0,17±0,03 (8)	0,54±0,18** (7)	0,09±0,03 (6)	0,07±0,00 (9)

Legend. Here and in Table 2, number of animals given in parentheses. *P < 0.01, **P < 0.05, ***P < 0.1.

TABLE 2. CAT Activity (in picomoles/μg protein/h) in Perifornical Region of Brain of Rabbits under Emotional Stress

Group of animals	CAT	P
Control 1	14,6±11,1 (6)	—
Control 2	31,8±7,9 (5)	<0,02
Predisposed rabbits	22,5±8,3 (6)	—
Resistant rabbits	26,7±4,9 (6)	<0,05

cardiovascular functions, surviving during the 3rd hour of the experiment (Table 2). CAT activity in the perifornical region of animals predisposed to stress-induced disorders did not differ significantly from control 1, whereas in animals with stable cardiovascular functions the level of activity was 183% of normal, which confirmed previous results [3]. The concentration of water-soluble proteins in all CNS structures studied was close to normal. In the ganglia of the autonomic nervous system a tendency was observed for the concentration of water-soluble proteins to fall, but only in SCG were significant changes observed.

This investigation of AChE and CAT activity showed that the intensity of cholinergic processes in the CNS structures of rabbits predisposed to cardiovascular disorders under emotional stress was indistinguishable from normal. Previously, in a study of animals with stable cardiovascular functions [3] comparable results were obtained for all CNS formations except the perifornical region of the hypothalamus (paraventricular and perifornical nuclei). In control 2 the level of CAT activity in the perifornical region, incidentally, differed considerably from normal. We know that even such weak stimuli as opening the cage, moving the animal into another room, and picking it up in the hand led to a sudden rise in the blood plasma catecholamine (CA) level [6]. It has been shown on the model used in the present investigation that scalping and immobilization of rabbits and insertion of electrodes into them evoke a stress reaction and elevation of the CA level in the adrenals and ganglia [4]. The results now obtained showed that the ACh system of the perifornical region of the hypothalamus responds just as dramatically to the preparatory stages of the experiment.

Unlike central brain formations, in structures of the autonomic nervous system appreciable differences in AChE level were found in animals predisposed to cardiovascular distur-

bances and resistant rabbits [3]. The rise of AChE activity in GN and SCG of rabbits toward the time of death and, consequently, increased activity of ganglia of the autonomic nervous system, could be achieved through total mobilization of the internal reserves: The reduction in the content of water-soluble proteins found in the ganglia indicates that catabolic processes were taking place in them.

Previously the writers postulated that participation of the cholinergic system of the perifornical region in maintenance of stability of cardiovascular functions during emotional stress may be connected with its regulatory influence on water and electrolyte metabolism [3]. This conclusion is in agreement with the results of the present series of experiments: In animals predisposed to cardiovascular disorders, in the absence of any marked shift in relations between components of the ACh system in structures of the CNS, including the perifornical region, there was no change in the content of water-soluble proteins (and also, evidently, of water) in the brain tissue.

This investigation showed that elevation of the BChE level both in the CNS and in ganglia of the autonomic nervous system (Table 1), is a parameter which characterizes the neurochemical organization of animals predisposed to cardiovascular disorders under emotional stress. BChE is an enzyme located in the brain parenchyma in glial cells, capillary walls, and the muscular layer of arterioles and venules. The mechanisms lying at the basis of functional changes in BChE activity are largely unexplained. Accordingly, the fact of a selective change in BChE activity in animals predisposed to cardiovascular failure under conditions of emotional stress, and the absence of changes in BChE activity in rabbits resistant with respect to the function tested, thus deserves attention.

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