# ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE IN THE NERVOUS SYSTEM OF RABBITS RESISTANT TO EMOTIONAL STRESS

N. V. Petrov, M. Fatranska, R. Kvetnansky, T. I. Belova, and S. I. Kashtanov

UDC 612.822.2.015.1:577.152.311/-06:613.863

KEY WORDS: emotional stress; resistance; perifornical region of the hypothalamus; acetylcholinesterase; choline acetyltransferase.

In the investigation described below the role of the cholinergic mediator system in the formation of resistance of the cardiovascular functions of animals to the harmful effect of a negative emotiogenic stimulus was studied. The intensity of cholinergic processes in a tissue is generally judged by the level of activity of enzymes of acetylcholine synthesis and breakdown. In this investigation activity of acetylcholinesterase (AChE), choline acetyltransferase (CAT), and also of butyrylcholinesterase (BChE) in brain nuclei and in ganglia of the autonomic nervous system was determined biochemically in rabbits with experimental emotional stress.

## EXPERIMENTAL METHOD

A state of emotional stress was induced in immobilized rabbits by stimulation of the ventromedial hypothalamic nuclei and by electrodermal stimulation [6]. Experiments were carried out on rabbits whose blood pressure varied negligibly. Slices of brain 300 μ thick were cut and the following formations were isolated as described in [12]: 1) the medullary reticular formation; 2) the mesencephalic reticular formation, dorsal portion; 3) mesencephalic reticular formation, ventral portion; 4) oral nucleus of the pons; 5) caudal nucleus of the pons; 6) ventromedial hypothalamic nucleus; 7) perifornical region; 8) preoptic region; 9) frontal cortex. Besides structures of the CNS, three ganglia of the autonomic nervous system also were studied: superior cervical and stellate sympathetic ganglia, ganglion nodosum of the vagus nerve. AChE activity was determined by Ellman's method [8]. By injecting inhibitor GD-42 in some tests at the beginning of incubation, BChE activity in the extract could be detected. In the perifornical region and superior cervical sympathetic ganglion CAT activity was determined by Fonnum's method, using labeled acetyl-CoA [9]. The perifornical region was chosen for CAT analysis deliberately: considering that positive correlation exists between the levels of AChE and CAT activity and a deficiency of $^{14}$C-acetyl-CoA, it was decided to study only a structure which showed changes in AChE activity. The content of water-soluble protein was determined by the method in [11].

## EXPERIMENTAL RESULTS

In rabbits with stable cardiovascular function AChE activity in most structures of the CNS tested and in ganglia of the autonomic nervous system was close to the control level (Table 1). Only in the perifornical region of the hypothalamus (paraventricular and perifornical nuclei and the adjacent part of the dorsomedial nucleus) was a tendency for AChE activity to rise observed — in the experimental animals its value was 167% of normal ($P < 0.1$). CAT activity in this structure was increased to 147% (Table 2). Under these conditions CAT activity in the superior cervical sympathetic ganglion was close to normal. In all samples of nerve tissue the content of water-soluble proteins was increased, and the dif-

P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Czechoslovakia. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 28-30, August, 1983. Original article submitted March 25, 1983.

0007-4888/83/9608-1059$07.50  

TABLE 1. AChE and BChE Activity and Protein Concentration in Structures of Nervous System of Rabbits in a State of Emotional Stress (M ± m)

| Structure | AChE, mmoles/min/mg protein | | Protein, mg/mg tissue | | BChE, mmoles/min/mg protein | |
|---|---|---|---|---|---|---|
| | control | experiment | control | experiment | control | experiment |
| Reticular formation | | | | | | |
| medullary | 0,23±0,06 (6) | 0,21±0,09 (5) | 0,03±0,005 (7) | 0,04±0,01** (5) | 0,03±0,01 (6) | 0,05±0,03 (6) |
| mesencephalic | 0,37±0,06 (6) | 0,33±0,08 (6) | 0,03±0,006 (7) | 0,04±0,01***(6) | 0,05±0,02 (6) | 0,06±0,04 (7) |
| dorsal region | | | | | | |
| ventral | 0,23±0,14 (6) | 0,29±0,16 (6) | 0,03±0,006(7) | 0,03±0,01***(6) | 0,06±0,02 (6) | 0,11±0,13 (7) |
| Oral nucleus of pons | 0,29±0,03 (6) | 0,25±0,14 (6) | 0,03±0,007 (7) | 0,03±0,01 (6) | 0,04±0,04 (6) | 0,11±0,16 (7) |
| Caudal nucleus of pons | 0,26±0,03 (6) | 0,21±0,05 (5) | 0,03±0,005 (7) | 0,03±0,002 (5) | 0,03±0,02 (6) | 0,06±0,03 (6) |
| Ventromedial hypothalamic nucleus | 0,11±0,07 (5) | 0,10±0,03 (6) | 0,06±0,006 (6) | 0,07±0,02 (6) | 0,01±0,01 (5) | 0,02±0,02 (7) |
| Perifornical region | 0,20±0,01 (6) | 0,32±0,10 (6)* | 0,04±0,005 (6) | 0,04±0,01 (6) | 0,02±0,02 (6) | 0,04±0,02 (7) |
| Preoptic region | 0,40±0,01 (6) | 0,45±0,29 (5) | 0,04±0,05 (6) | 0,04±0,01** (5) | 0,03±0,02 (6) | 0,03±0,03 (6) |
| Cortex | 0,12±0,03 (5) | 0,14±0,09 (6) | 0,03±0,003 (6) | 0,04±0,003**(6) | 0,02±0,01 (6) | 0,05±0,04 (7) |
| Superior cervical ganglion | 1,53±0,35 (7) | 1,49±0,45 (7) | 0,07±0,02 (8) | 0,08±0,01 (7) | 1,08±0,36 (7) | 1,39±0,34 (8) |
| Stellate ganglion | 1,32±0,23 (7) | 1,34±0,51 (7) | 0,07±0,01 (8) | 0,08±0,01 (7) | 0,59±0,46 (7) | 0,97±0,58 (8) |
| Ganglion nodosum | 0,42±0,21 (6) | 0,41±0,28 (7) | 0,07±0,04 (7) | 0,10±0,02 (8) | 0,14±0,13 (7) | 0,13±0,04 (8) |

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

TABLE 2. CAT Activity (in pmoles/mg protein/h) in Structures of Nervous System in Rabbits in a State of Stress (M ± m)

| Structure | Control | Experiment |
|---|---|---|
| Perifornical region | 15,7±1,9 (6) | 23,1±2,2** (6) |
| Superior cervical ganglion | 24,9±1,8 (3) | 29,2±2,1 (6) |

**P < 0.05.

ferences were significant in the medullary reticular formation, the mesencephalic reticular formation (dorsal and ventral portions), the preoptic region, and cortex. The level of BChE activity in the structures tested in the experimental animals was close to the control level, i.e., BChE did not correlate significantly with the animal's state during stress.

The investigation showed that of all the brain formations studied, and the autonomic nervous system, it was in the perifornical region that the cholinergic mechanism plays an active part in the regulation of cardiovascular functions in emotional stress. Data on changes in AChE and CAT activity in this region agree with the results of previous histochemical studies on immobilized rats [2]. There is evidence that the cholinergic mediator system of the hypothalamus plays an essential role in the development of the stressor response [1, 4]. Experiments have shown that neurosecretion is potentiated during activation of cholinergic elements of the hypothalamus.

Participation of the perifornical region in feeding and drinking behavior has been demonstrated experimentally. Experiments on rats have shown that during dehydration of the body a decrease in the content of neurosecretion, hypertrophy of the neurons, and enlargement of the nucleus and nucleolus are observed in the paraventricular nucleus [3]. The morphological changes discovered can be regarded as a sign of enhanced activity of the paraventricular nucleus [10]. Release of neurosecretion in the paraventricular nucleus during dehydration is evidently due to activation of cholinergic processes; experiments on rabbits have shown that dehydration is accompanied by increased AChE activity in the paraventricular nucleus [5]. It can be concluded from a comparison of the results of the writers' own observations with data in the literature that the increase in acetylcholine metabolism discovered in the present investigation may be connected with dehydration of the body during stress. Evidence in support of dehydration is given by the increase in the content of water-soluble proteins in all samples of nerve tissue tested in this investigation (Table 1).

There is evidence that dehydration and accompanying changes in tissue metabolism determine the resistance of the body to the action of unfavorable factors [7]. The participation of the cholinergic system of the perifornical region of the hypothalamus in maintaining resistance of cardiovascular functions in stress may be to some degree linked with its regulatory effect on water and electrolyte metabolism.

## LITERATURE CITED


1. L. Kh. Allikmets, in: Structural, Functional, and Neurochemical Organization of Emotions [in Russian], Leningrad (1971), pp. 144-147.
2. T. I. Belova, in: Current Problems in Physiology [in Russian], Moscow (1976), pp. 192-229.
3. A. A. Voitkevich and G. A. Ovchinnikova, Byull. Éksp. Biol. Med., No. 1, 93 (1962).
4. P. P. Denisenko, in: Stress and Its Pathogenetic Mechanisms [in Russian], Kishinev (1973), pp. 20-22.
5. T. A. Mikhailik, Byull. Éksp. Biol. Med., No. 12, 734 (1978).
6. N. V. Petrova, S. I. Kashtanov, and V. V. Portugalov, Patol. Fiziol., No. 1, 42 (1983).
7. L. V. Serova, I. A. Langemak, G. M. Potapova, et al., in: Stress and Its Pathogenetic Mechanisms [in Russian], Kishinev (1973), pp. 36-37.
8. J. L. Ellman, Biochem. Pharmacol., 7, 88 (1961).
9. F. Fonnum, J. Neurochem., 24, 407 (1975).
10. R. C. Holland, B. A. Cross, and C. H. Sawyer, Am. J. Physiol., 196, 791 (1959).
11. M. Palkovits, in: Anatomical Neuroendocrinology, Basel (1975), pp. 72-80


# EFFECT OF COOLING ON MECHANISMS OF MYOCARDIAL CONTRACTILITY AUTOREGULATION IN WARM-BLOODED ANIMALS


V. M. Pokrovskii, V. V. Vovereidt, and Yu. R. Sheikh-Zade



UDC 612.172-06:612.592

KEY WORDS: cat's heart; autoregulation of contractility; cooling.


Starling's mechanism (dependence of the force of contraction of heart muscle on its length) and the chrono-inotropic mechanism (dependence of force on the frequency of contractions) are the fundamental myogenic mechanisms of autoregulation of myocardial contractility. However, no unequivocal answer has yet been given to the question of the effect of a factor such as cooling on these mechanisms. Some workers, for instance, have concluded that functioning both of Starling's mechanism and of the chrono-inotropic mechanism is improved with a fall of myocardial temperature [1, 6-8]. Other experimental studies, however, contradict this conclusion [9-11].

In the investigation described below the effect of temperature was studied on the efficiency of stretching and on the increase in heart rate connected with regulation of myocardial contractility in warm-blooded animals.

## EXPERIMENTAL METHOD

Experiments were carried out on papillary muscles 4-8 mm long and not more than 1 mm in diameter, from the right ventricle of a cat's heart. The preparation was placed in a constant temperature chamber where one end was fixed to the stretching device, the other end to a 6MKh1S mechanotron force transducer [2]. The preparation was immersed in modified Krebs–Henseleit solution (in mM): NaCl 118.0, KCl 2.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.0, $MgSO_4$ 1.2, $NaHCO_3$ 12.0, glucose 5.6. The solution was saturated with a gas mixture of 95% $O_2$ and 5% $CO_2$; the pH of the solution was 7.3-7.4. The muscle was stimulated by above-threshold square


Department of Normal Physiology, Red Army Kuban Medical Institute, Krasnodar. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.)


Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 30-33, August, 1983. Original article submitted March 19, 1982.