LITERATURE CITED

1. J. Chamley-Campbell, G. R. Campbell, and R. Ross, Physiol. Rev., 59, 1 (1979).
2. E. Chung and E. J. Miller, Science, 183, 1200 (1974).
3. J.-A. Grimaud, M. Druguet, S. Peyrol, et al., J. Histochem. Cytochem., 28, 1145 (1980).
4. A. V. Krushinsky and A. N. Orekhov, in: Vessel Wall in Athero- and Thrombogenesis: Studies in USSR, E. I. Chazov and V. N. Smirnov, eds., Berlin (1982), p. 41.
5. K. G. McCullagh and G. Balian, Nature, 258, 73 (1975).
6. K. G. McCullagh, V. C. Duance, and K. A. Bishop, J. Pathol., 130, 45 (1980).
7. H. Nowack, S. Gay, G. Wick, et al., J. Immunol. Methods, 12, 117 (1976).
8. A. N. Orekhov, V. A. Kosykh, and A. V. Pokrovskii, in: Vessel Wall in Athero- and Thrombogenesis: Studies in USSR, E. I. Chazov and V. N. Smirnov, eds., Berlin (1982), p. 53.
9. K. Peitila and T. Nikkari, Atherosclerosis, 37, 11 (1980).
10. J. Rauterberg, S. Allam, U. Brehmer, et al., Hoppe-Seylers Z. Physiol. Chem., 358, 401 (1977).
11. R. Ross and S. J. Klebanoff, J. Cell Biol., 50, 159 (1971).
12. S. B. Russell, J. D. Russell, and K. M. Trupin, J. Cell Physiol., 109, 121 (1981).
13. H. Sage, R. G. Woodbury, and P. Bornstein, J. Biol. Chem., 254, 9893 (1979).
14. R. L. Treslad, Biochem. Biophys. Res. Commun., 57, 717 (1974).
15. T. H. Weller and A. H. Coons, Proc. Soc. Exp. Biol. (N.Y.), 86, 789 (1954).

MONOAMINE OXIDASE ACTIVITY IN SYMPATHETIC GANGLIA OF RABBITS DIFFERING IN RESISTANCE OF THEIR CARDIOVASCULAR FUNCTIONS TO EMOTIONAL STRESS

A. V. Gorbunova and S. I. Kashtanov

UDC 616.834-008.931:577.152.143/-02:613.863-092.9

KEY WORDS: catecholamines; hypothalamus; rabbits.

The study of catecholamines and the enzymes of their metabolism in structures of the peripheral nervous system during stress is of great interest. It was shown previously [3] that changes in activity of tyrosine hydroxylase, a key enzyme of catecholamine biosynthesis, in the ganglion nodosum and stellate ganglion differ in rabbits "predisposed" and "resistant" to emotional stress. In rabbits "predisposed" to stress a marked decrease in tyrosine hydroxylase activity was observed in the stellate ganglion and ganglion nodosum, whereas in rabbits "resistant" to stress tyrosine hydroxylase activity in the stellate ganglion and ganglion nodosum did not differ from the control level.

The object of this investigation was to study activity of monoamine oxidase (MAO), an enzyme inactivating catecholamines in neurons of the superior cervical and stellate ganglia and the ganglion nodosum in rabbits differing in the resistance of their cardiovascular functions to emotional stress.

EXPERIMENTAL METHOD

Emotional stress was induced in immobilized mature Chinchilla rabbits by simultaneous aperiodic stimulation of the negative emotiogenic centers of the hypothalamus (ventromedial nuclei) and electrodermal stimulation according to a specially devised stochastic scheme. The ventromedial hypothalamic nuclei were stimulated by bipolar nichrome electrodes, and electrodermal stimulation was applied through steel needles, implanted subcutaneously into one of the animal's hind limbs. The parameters of the electric current (square pulses: frequency 50 Hz, pulse duration 1 msec, voltage 5-10 V, duration of stimulation 1-2 min) were

B. I. Lavrent'ev Laboratory of Neurohistology and Electron Microscopy Cabinet, P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow.
(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. 10, pp. 111-113, October, 1983. Original article submitted March 25, 1983.

 0007/4888/83/9610-1476$07.50

chosen individually with a view to causing the blood pressure (BP) to rise by 20-30 mm Hg without any marked motor reaction of the animal. Throughout the period of realization of the stress program (3 h) the rabbits' BP was recorded continuously by means of strain-gauge and piezoelectric transducers connected to a Mingograf (Siemens-Elema, Sweden) recorder. Depending on the character of the changes in BP the experimental animals were divided into two groups: "resistant" and "predisposed" to stress. The "resistant" animals were characterized by very small fluctuations of BP, whereas in rabbits "predisposed" to stress BP fell progressively until death took place 2 h 15 min after the beginning of the experiment. The ganglion nodosum (of the vagus nerve) and the cervical and stellate (sympathetic) ganglia were removed immediately after death of the rabbits "predisposed" to stress and, in addition, the corresponding structures were taken from rabbits killed by air embolism 3 h after the beginning of the experiment. Animals kept under ordinary conditions in the animal house served as the control. MAO activity was detected by Glenner's method [10] in sections (20 μ) of frozen (in a cryostat at −18°C) unfixed tissue. Air-dried (for 3 min) sections were incubated in 20 ml of medium containing 25 mg tryptamine hydrochloride (from Reanal, Hungary), 5 ml of 0.1 M phosphate buffer, pH 7.6, 5 mg nitro-BT (from Chemapol, Czechoslovakia), 4 mg sodium sulfate, and 15 ml distilled water for 40 min at 37°C. As the control, tissue sections were treated in the absence of the enzyme substrate. After incubation the sections were fixed in 10% neutral formalin at room temperature for 20 min and mounted in glycerol-gelatin. For quantitative evaluation of the histochemical reaction for MAO the method [1] was used; this is based on discrete measurement of the density of diformazan (formed as a result of reduction of nitro-BT during oxidative deamination of MAO substrates) in the sections on a LYUMAM (from LOMO, USSR) microscope with photometric attachment, at a wavelength of 590 nm, in which according to data in the literature [1] the maximum of absorption of sections after Glenner's reaction falls. Photometry was carried out with a 40× objective and 7× ocular and the diameter of the probe was 0.1 mm. MAO activity, taken to be equal to the density of formazan deposition in the sections, was expressed in relative units and calculated by the equation $E = \log (F_0/F)$, where F is the photic flux passing through the preparation and F_0 the flux passing through the preparation close to the section. Measurements were made in cells with clearly distinguishable nucleus and nucleolus. Measurements were made at five points of the cytoplasm around the nucleus in each cell. The numerical data were subjected to statistical analysis by the Student–Fisher method [6].

EXPERIMENTAL RESULTS

In rabbits "resistant" to stress a significant increase in MAO activity compared with the control was observed in neurons of the cervical and stellate ganglia, whereas in neurons of the ganglion nodosum MAO activity was the same as the control level (Table 1). In rabbits "predisposed" to stress MAO activity in neurons of the stellate and cervical ganglia was significantly below the control level, whereas in the ganglion nodosum it was the same as in the control.

An increase in activity of the sympathicoadrenal system under conditions of emotional stress [4, 5], accompanied in all probability by increased release of noradrenalin from terminals of adrenergic neurons, must now be regarded as firmly established. A considerable increase in the blood catecholamine concentrations has been observed under these circumstances [5,9], i.e., the catecholamine level rises in the internal medium of the body. One of the main mechanisms of inactivation of noradrenalin released into the blood stream is its reuptake by adrenergic neurons, as a result of which the noradrenalin concentration may rise in cells of the stellate and cervical ganglia. This hypothesis is supported by data obtained by Portugalov et al. [7], who observed an increase in the noradrenalin concentration in homogenates of the stellate (experiment 3880 ng, control 1870 ng) and cervical (experiment 5381 ng, control 5152 ng) ganglia of rabbits "predisposed" to stress, toward the end of an experiment lasting 3 h, when tyrosine hydroxylase activity in these structures was depressed (by 49% in the superior cervical and by 66% in the stellate ganglion) [3]. Intraganglionic release of catecholamines by small, intensely fluorescent cells could also lead to an increase in the noradrenalin concentration in the neurons [12]. The increase in the intracellular catecholamine concentration is accompanied as a rule by more intensive inactivation of catecholamines as a protective reaction of the body, and one which probably took place under the present experimental conditions in rabbits "resistant" to stress, in which an increase in MAO activity was observed in the neurons studied. In all probability stabilization of catecholamine anabolism and catabolism takes place at a new level corresponding to the conditions of stress, and this hypothesis is supported by the absence of changes, established previously [3], in tyrosine hydroxylase

TABLE 1. MAO Activity in Neurons of Sympathetic and Parasympathetic Ganglia (M ± m)

Ganglion	Control (number of animals 9, number of neurons 360)	Rabbits "predisposed" to stress (number of animals 4, number of neurons 160)	P	Rabbits "resistant" to stress (number of animals 5, number of neurons 200)	P
Nodosum	0,31±0,003	0,32±0,003		0,31±0,0044	
Superior cervical	0,72±0,005	0,70±0,006	<0,05	0,77±0,0056	<0,001
Stellate	0,84±0,006	0,80±0,0087	<0,01	0,88±0,006	<0,001

activity in the stellate ganglion and ganglion nodosum and an increase in MAO activity in neurons of the cervical and stellate ganglia under the present experimental conditions. The noradrenalin concentration remained high [7] in neurons of the cervical and stellate ganglia of rabbits "predisposed" to stress, in which MAO activity was depressed.

On the one hand, this could lead to modification of the enzyme tyrosine hydroxylase, i.e., to a change in its kinetic properties [8], manifested as a reduction of its activity [3]. On the other hand, high concentrations of noradrenalin, acting on α-adrenoreceptors located on adrenergic endings, may have inhibited any further release of mediator [2]. All these events probably led to a disturbance of sympathetic transmission and, as a result, of the nutrition and activity of the heart in rabbits "predisposed" to stress.

A change in activity of MAO, the enzyme of catecholamine breakdown, was thus observed in neurons of sympathetic ganglia (cervical, stellate) during acute experimental emotional stress; in rabbits "resistant" to stress, moreover, MAO activity was observed to be increased, whereas in animals "predisposed" to stress activity was reduced. In the ganglion nodosum of the vagus nerve MAO activity remained unchanged under conditions of acute experimental stress.

LITERATURE CITED

1. A. Yu. Budantsev, Monoaminergic Brain Systems [in Russian], Moscow (1976).
2. D. Burnstock and M. Costa, Adrenergic Neurons: Their Organization, Function, and Development in the Peripheral Nervous System [Russian translation], Minsk (1979).
3. A. V. Gorbunova, V. S. Kudrin, S. I. Kashtanov, et al., Byull. Éksp. Biol. Med., No. 8, 22 (1982).
4. G. N. Kassil', in: Current Problems in Stress [in Russian], Kishinev (1976), p. 100.
5. É. Sh. Matlina, Usp. Fiziol. Nauk, No. 3, 92 (1972).
6. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
7. V. V. Portugalov, N. V. Petrova, S. I. Kashtanov, et al., Patol. Fiziol, No. 5, 28 (1981).
8. K. S. Raevskii, M. F. Mineeva, and V. S. Kudrin, in: Catecholaminergic Neurons [in Russian], Moscow (1979), p. 232.
9. L. S. Ul'yaninskii, E. P. Stepanyan, and E. N. Agapova, Kardiologiya, No. 4, 94 (1978).
10. G. G. Glenner, H. J. Burtner, and G. W. Brown, J. Histochem. Cytochem., 5, 591 (1957).
11. S. L. Iverson, Biochem. Pharmacol., 23, 1927 (1974).
12. T. H. Williams and S. L. Palay, Brain Res., 15, 17 (1969).