INVESTIGATION OF MONOAMINE OXIDASE ACTIVITY IN THE TISSUES OF RABBITS EXPOSED TO ACUTE STRESS

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Monoamine oxidase activity of the heart and liver relative to tyramine and of the brain relative to tyramine and serotonin was studied in experiments on rabbits exposed to acute stress by immobilization. The catecholamine concentration was determined simultaneously in the adrenal, heart, liver, and brain. The catecholamine level in the tissues of the experimental animals was found to be lower than in the control. Monoamine oxidase activity of the liver and heart was not significantly changed. In the brain of the rabbit exposed to stress, changes in the activity of this enzyme occurred in different directions: it was decreased relative to tyramine but increased relative to serotonin.

Investigations [8, 9, 11, 13] have shown that the catecholamine level is lowered in the adrenals, brain, heart, and liver of animals with experimental stress. It is interesting to study the pathways of catecholamine metabolism under these conditions. In certain chronic stress states the writer has found [2] an increase in the volume of the quinoid conversion of catecholamines. As a result the catecholamines are not completely inactivated, but products are formed with a marked catalytic and pharmacodynamic action [5, 6, 7].

The object of the present investigation was to study activity of mitochondrial monoamine oxidase (MAO), an enzyme participating in metabolism of the biogenic monoamines (including the catecholamines) in rabbits exposed to acute stress by immobilization. The catecholamine concentration was studied simultaneously in the adrenal, brain, heart, and liver.

EXPERIMENTAL METHOD

Altogether 25 chinchilla rabbits of both sexes (weight 2-2.5 kg) were used. The state of stress was induced by binding the animals to the operating table in the supine position for 10 min.

Mitochondria of the heart, liver, and brain were sedimented by differential centrifugation in isotonic sucrose solution [15]. Mitochondrial MAO activity was determined from the quantity of ammonia formed during incubation of suspensions of mitochondria in 0.24 M phosphate buffer, pH 7.4 [1], with tyramine as substrate in a Warburg apparatus at 38°C for 60 min. For the brain, serotonin also was used as substrate. The substrates were added at the rate of 10 μmoles per sample. At the end of incubation, 10% trichloroacetic acid was added (1:1) to the samples, and after centrifugation, the ammonia concentration was determined in the supernatant by Conway's isothermic distillation method followed by nesslerization. MAO activity was expressed in μg ammonia/mg protein. Protein was determined by the method of Lowry et al. [12]. To determine catecholamines and products of their quinoid oxidation, Osinskaya's trihydroxyindole fluorescence method was used. The catecholamine concentration was expressed in micrograms per organ, while in the adrenal the calculation was made with respect to one of the paired organs (the left). The control group

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TABLE 1. Effect of Acute Stress on Catecholamine Concentration in Organs of Rabbits

Organ	Catecholamine	Catecholamine content (in μg)		P
		control group	experimental group	
Adrenal (left)	Adrenalin	39,7±3,1 (n=32)	16,6±1,61 (n=11)	<0,01
Brain	Noradrenalin	2,15±0,16 (n=27)	1,25±0,18 (n=12)	<0,05
Heart	»	7,25±0,22 (n=27)	3,00±0,31 (n=12)	<0,01
Liver	»	45,0±9,1 (n=28)	24,7±4,0 (n=11)	<0,05

TABLE 2. Mitochondrial MAO Activity in the Brain of Rabbits with Acute Stress

Substrate	MAO activity (in μg NH_3/mg protein)		P
	control group	experimental group	
Serotonin	2,53±0,13 (n=11)	3,00±0,18 (n=12)	<0,05
Tyramine	5,02±0,1 (n=11)	4,4±0,17 (n=11)	<0,05

consisted of 32 intact rabbits. The experimental results were subjected to statistical analysis using Student's tables.

EXPERIMENTAL RESULTS

A marked decrease in catecholamine concentration was found in the organs of the experimental rabbits compared with the control (Table 1). Substances with the properties of products of quinoid oxidation of catecholamines in acute stress were found only in the liver, but their level and the frequency of their appearance did not exceed the control. By comparison with earlier findings obtained in the study of certain types of chronic stress [3], during exposure to acute stress the volume of quinoid conversion evidently was not increased.

The mitochondrial MAO activity of the liver and heart of the experimental animals relative to tyramine was not significantly changed, being 16.4 ± 0.7 μg/NH_3 mg protein for the liver compared with the normal value of 17.0 ± 0.22 μg/mg, while the corresponding figures for the heart were 1.55 ± 0.055 μg/mg compared with a normal 1.47 ± 0.085 μg/mg. This suggests that deamination does not play an essential role in catecholamine conversion in the liver and heart during exposure to acute stress. Under those conditions the role of other metabolic pathways (possibly, methoxylation), evidently is more important.

During incubation of the brain mitochondrial preparations with tyramine and serotonin, changes in MAO activity occurred in different directions with the two substrates. Deamination of tyramine in the experimental animals was reduced compared with the control; with respect to serotonin the activity of the enzyme was increased (Table 2). The decrease in brain MAO activity with respect to tyramine led to an increase in the content of this amine in the brain, and this in turn affected the nonliberation of noradrenalin from the granules, thereby controlling its level [10].

Experiments [14] have shown that in acute stress the serotonin level in the brain rises; an increase in MAO activity relative to serotonin thus leads to its rapid inactivation and prevents its excessive accumulation.

The changes in MAO activity of the brain with respect to tyramine and serotonin found in these experiments are evidently compensatory-adaptive in character.

LITERATURE CITED

1. S. D. Balakhovskii and I. S. Balakhovskii, Methods of Chemical Analysis of Blood [in Russian], Moscow (1953), p. 318.
2. G. A. Loginova, Investigation of Certain Processes of Catecholamine Metabolism in States of Stress. Author's Abstract of Candidate's Dissertation, Khar'kov (1969).
3. G. A. Loginova, Collected Scientific Transactions of Khar'kov Medical Institute [in Russian], No. 78, Khar'kov (1968), p. 83.
4. V. O. Osinskaya, Biokhimiya, No. 3, 537 (1957).

5. A. M. Utevskii, Uspekhi Sovr. Biol., 18, 145 (1944).
6. A. M. Utevskii, Probl. Éndokrinol., No. 1, 19 (1955).
7. A. M. Utevskii, V. O. Osinskaya, and P. A. Kaliman, Ukr. Biokhim. Zh., No. 5, 798 (1965).
8. D. Barchas and D. X. Freedman, Biochim. Pharmacol., 12, 1232 (1963).
9. R. Gordon, S. Spector, A. Sjördsma, et al., J. Pharmacol. Exp. Ther., 153, 440 (1966).
10. J. Kopin and E. Gordon, J. Pharmacol. Exp. Ther., 138, 351 (1962).
11. R. Kvetńansky and L. Miculaj, Endocrinology, 87, 738 (1962).
12. O. H. Lowry, N. Rosebrough, and A. Farr, J. Biol. Chem., 193, 265 (1951).
13. L. Lubańska-Tomaszewska, Acta Physiol. Pol., 15, 831 (1964).
14. M. Ruckebusch and C. Brunet-Tallon, C. R Soc. Biol. (Paris), 160, 2131 (1966, 1967).
15. W. C. Schneider, J. Biol. Chem., 176, 259 (1948).