9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 256 (1951).
10. S. T. Pacold, S. L. Kirstein, S. Hojvat, et al., Science, 199, 804 (1978).
11. S. Payne and J. L. Kostyo, Endocrinology, 87, 1186 (1970).
12. V. P. Whittaker et al., Biochem. J., 90, 239 (1964).

EFFECT OF LOW TEMPERATURE ON ACTIVITY AND SUBSTRATE SPECIFICITY OF MONOAMINE OXIDASES IN RAT BRAIN MITOCHONDRIA

I. A. Goroshinskaya, A. A. Krichevskaya, and Z. G. Bronovitskaya

UDC 612.58:612.822.1.015.1:577.152.143

Modification of the activity and properties of enzymes is one of the more important components of adaptive changes to low temperature at the molecular level. The leading role of neurohumoral mechanisms of regulation of adaptation to cold [11, 14] explains the current importance of the study of the effect of low temperatures on mitochondrial monoamine oxidase [monoamine:oxygen-oxidoreductase (deaminating) — MAO], which is of fundamental inportance to metabolism of the monoamines and the performance of their mediator functions. Two forms of the enzyme are distinguished: type A MAO, inhibited by chlorgyline, substrates of which are serotonin and noradrenalin, and type B MAO, inhibited by deprenil, substrates of which are β-phenylethylamine and benzylamine [15].

The object of this investigation was to study the activity and substrate specificity of MAO of types A and B under conditions of cold stress.

EXPERIMENTAL METHOD

Albino rats weighing 150-180 g were kept in a cold room at 2°C for 3 days. Under these circumstances the animals' rectal temperature remained unchanged. Keeping the animals at low temperatures for 3 days induced marked manifestations of a stress reaction in them the outflow of noradrenalin from the hypothalamus and sympathetic nerve endings, the secretion of catecholamines and corticosteroids from the adrenals, and so on, were increased [1, 9, 14]. Rats of the same age and body weight, kept in the animal house at 20-22°C, served as the control. The animals were decapitated in the cold room and all subsequent operations were performed in the cold. The brain was homogenized in 0.25 Msucrose solution made up in0.02 M phosphate buffer, pH 7.45. Mitochondria were isolated by differential centrifugation [10]. Deamination of serotonin, noradrenalin, glucosamine, AMP, putrescein, and γ-aminobutyric acid (GABA) was judged from the liberation of ammonia after incubation of the suspension of mitochondria with one of the substrates in saturating concentration. Incubation was carried out in a medium of air at 37.5°C and pH 7.45 for 30 min. The ammonia content was determined by the phenol and hypochlorite method after isothermic distillation [4]. When p-nitrophenylethylamine was used as the substrate, MAO activity was judged from the intensity of the color which developed as a result of condensation of the aldehyde, formed under the influence of the enzyme, with excess of substrate [10]. Protein was determined by Lowry's method. When the effect of chloryline and deprenil on MAO activity was studied the suspension of mitochondria was preincubated in one of the inhibitors for 15 min at 20°C. The work was done in January-March, which is important in connection with data showing seasonal differences in the response of animals to cold [13].

EXPERIMENTAL RESULTS

As Table 1 shows, keeping the animals for 3 days at 2°C led to a significant fall in type A MAO activity: The intensity of deamination of serotonin was reduced by 32% and of noradrenalin by 29%. The decrease in MAO activity with natural substrates was accompanied by a

KEY WORDS: cooling; brain; monoamine oxidase.

Laboratory of Mechanisms of Ecologic Adaptation of Animals to Extremal Environmental Factors, Biological Research Institute, Rostov-on-Don University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 431-433, April, 1981. Original article submitted May 21, 1980.

 0007-4888/81/9104-0464$07.50

TABLE 1. Deamination of Nitrogenous Compounds by Rat Brain Mitochondria under the Influence of Cold (in nmoles ammonia nitrogen/mg protein/min, M ± m)

Experimental conditions	Serotonin	Substrate					
		noradrenalin	glucosamine	AMP	putrescein	GABA	p-nitrophenyl-ethylamine
Control	7,17±0,42 (12)	5,46±0,44 (13)	0,54±0,24 (13)	2,46±0,35 (13)	0,57±0,21 (13)	0,22±0,08 (13)	3,43±0,16 (14)
Males	7,54±0,31 (5)	5,94±0,57 (6)	0,62±0,45 (6)	2,70±0,64 (6)	0,47±0,32 (6)	0,18±0,12 (6)	3,34±0,23 (6)
Females	6,91±0,68 (7)	5,04±0,68 (7)	0,47±0,27 (7)	2,26±0,39 (7)	0,65±0,30 (7)	0,25±0,16 (7)	3,52±0,23 (8)
Cold	4,85±0,52 (12)	3,85±0,65 (12)	4,51±0,54 (10)	5,48±0,65 (10)	1,43±0,34 (10)	1,31±0,39 (10)	3,20±0,21 (10)
P	<0,01	<0,05	<0,001	<0,001	<0,05	<0,01	
Percent of change	−32	−29	+ 735	+ 123	+ 151	+ 495	
Males	5,36±0,69 (6)	4,39±1,10 (6)	4,48±0,90 (5)	4,21±0,80 (5)	1,39±0,70 (5)	1,31±0,74 (5)	3,01±0,22 (6)
Percent of change	−29	−26	+ 623	+ 56	+ 196	+ 628	
Females	4,34±0,78 (6)	3,32±0,93 (6)	4,55±0,72 (5)	6,32±0,69 (5)	1,47±0,35 (5)	1,32±0,38 (5)	3,58±0,45 (4)
Percent of change	−37	−34	+ 868	+ 180	+ 126	+ 428	

Legend. Number of experiments shown in parentheses. Percentage of change under the influence of cold calculated relative to control of the corresponding sex.

marked increase in the intensity of deamination of glucosamine, AMP, putrescein, and GABA. It should be pointed out that a decrease in the degree of deamination of serotonin was not observed in all animals in response to brief exposure to cold. In experiments in the same season of the previous year the intensity of serotonin deamination fell only slightly and in only half of the animals, whereas in the rest the deamination of serotonin was increased. Changes in the intensity of deamination of the other substrates were similar to those discovered in the present investigation. Unlike type A MAO, activity of type B MAO, judged by deamination of p-nitrophenylethylamine [5], was unchanged after exposure to cold for 3 days. Comparison of the responses of males and females to cold showed that activity of the enzyme with all substrates changed in the same direction in animals of the two sexes. The degree of inhibition of type A MAO and the intensity of deamination of unusual substrates under the influence of cold was a little higher in females than in males.

The results suggest that during exposure to cold qualitative changes take place in the catalytic properties of MAO, similar to those found previously in radiation sickness, malignant tumors, hypervitaminosis D_2, hyperoxia, and various other pathological conditions, due to transformation of the enzyme [2, 3, 6].

To confirm this hypothesis and to investigate which form of MAO is responsible for the appearance of ability to deaminate unusual substrates under the influence of cold, the effect of preincubation of the enzyme with selectively acting inhibitors — chlorgyline and deprenil — was studied; these inhibitors were used in a concentration of 10^{-6} M, in which they exert their most selective action [5, 15]. It will be clear from Fig. 1 that chlorgyline, which depressed serotinin deamination by 92%, prevented the deamination of glucosamine, putrescein, and GABA. Deprenil, which inhibits activity of type B MAO practically completely, did not reduce the deamination of serotonin or of unusual substrates. The intensity of deamination of AMP was unchanged both by chlorgyline and by deprenil. Consequently, stimulation of deamination of glucosamine, putrescein, and GABA by brain mitochondria under the influence of cold is due to a change in the substrate specificity of type A MAO, whereas stimulation of deamination of AMP is unconnected with MAO and is possibly due to activation of adenylate deaminase, responsible for the parallel transformation of MAO found in alcoholic intoxication [7].

Changes in the catalytic properties of MAO in other pathological conditions are associated with oxidation of enzyme SH groups by lipid peroxides [2, 3]. It can be tentatively suggested that the same mechanism of change in the catalytic properties of MAO is found also in cold stress. During the first day of the animals' stay in the cold the intensity of lipid peroxidation in the brain was increased, to reach a maximum on the 3rd day [8].

The results of this investigation suggest an important role for the change in activity and substrate specificity of MAO in the response of the animal to cold. An increase in functional activity of the sympathicoadrenal system, accompanied by an increase in noradrenalin

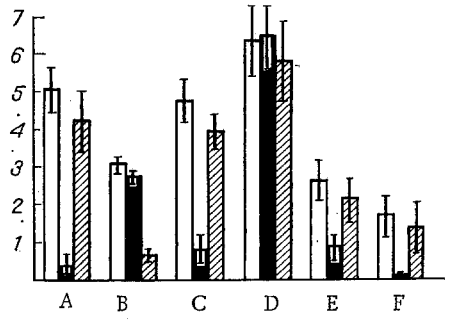

Fig. 1. Effect of MAO inhibitors on deamination of some nitrogenous compounds by brain mitochondria under the influence of cold. Unshaded columns — control, black columns — chlorgyline, obliquely shaded columns — deprenil. A) Serotonin; B) p-nitrophenylethylamine; C) glucosamine; D) AMP; E) putrescein; F) GABA. Ordinate, intensity of deamination (in nmoles ammonia nitrogen/mg protein/min.

liberation and in the efficiency of noradrenergic transmission, is regarded as one of the most important factors in adaptation to cold [11, 14]. The fall in the intensity of deamination or noradrenalin, leading to elevation of its concentration, is evidently one element of the adaptive changes in metabolism. A change in the substrate specificity of MAO may be one cause of the lowering of the polyamine level observed under the influence of cold [12], for transformed MAO acquires the ability to deaminate polyamines as well as other substances [2]. The change in substrate specificity of MAO can thus be regarded as an additional mechanism of regulation of the enzyme during a change in environmental conditions.

LITERATURE CITED

1. A. A. Ardashev, V. V. Semikina, and A. F. Mosin, in: Neuroendocrine Correlations [in Russian], Vladivostok (1978), p. 77.
2. V. Z. Gorkin. Vop. Med. Khim., 18, 118 (1972).
3. V. Z. Gorkin, Mol. Biol., 10, 717 (1976).
4. V. Z. Gorkin, I. V. Verevkina, L. I. Gridneva, et al., in: Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1968), p. 155.
5. I. A. Goroshinskaya, Vop. Med. Khim., 25, 328 (1979).
6. I. A. Goroshinskaya and Z. G. Bronovitskaya, Vop. Med. Khim., 22, 558 (1976).
7. G. D. Isakhanyan and V. Z. Gorkin, Vop. Med. Khim., 22, 76 (1976).
8. L. V. Lomakina, Ukr. Biokhim. Zh., 52, 305 (1980).
9. E. V. Maistrakh, Fiziol. Zh. SSSR, 65, 1582 (1979).
10. T. A. Moskvitina, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), p. 22.
11. Yu. F. Pastukhov and V. V. Khaskin, Usp. Fiziol. Nauk, No. 3, 121 (1979).
12. Y. Habara, T. Kanno, and A. Saito, J. Physiol. (London), 293, 447 (1979).
13. E. Preston and E. Schönbaum, in: Brain Dysfunction in Infantile Convulsions, New York (1976), p. 75.
14. H.-Y. Yang and N. H. Neff, J. Pharmacol. Exp. Ther., 187, 365 (1973).