A POSSIBLE MECHANISM OF CHANGES IN THE CATALYTIC PROPERTIES OF RAT BRAIN MONOAMINE OXIDASE

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Monoamine oxidase (MAO) is a key enzyme in the metabolism of many very important biogenic amines and largely determines their level in the tissues, and it also performs mediator and hormonal functions. Two forms of the enzyme are distinguished: MAO of type A (inhibitor chlorgyline, principal substrates serotonin and noradrenalin) and MAO type B (inhibitor deprenyl, substrates benzylamine, β-phenylethylamine, and methylhistamine) [11, 13]. In several pathological states accompanied by stimulation of lipid peroxidation, such as radiation injuries, the development of malignant tumors, hypervitaminosis D_2, and experimental hypercholesterolemia qualitative changes take place in the catalytic properties of MAO, manifested as a decrease in monoamine deaminase activity and the appearance of ability to deaminate unusual substrates [1, 7]. Similar changes in activity and substrate specificity were found by the writer in hyperoxia, hypoxia, and cold stress, and the role of these changes in the response of the body to these extremal factors was demonstrated. In all these states type A MAO activity is reduced in the mitochondrial fraction of rat brain, and this form of the enzyme becomes able to deaminate glucosamine, putrescine, and γ-aminobutyric acid [3-5].

Changes in MAO activity may be due, first, to changes in the molecular properties of the enzyme, which would be reflected in the reaction kinetics, and second, to changes in the structure and permeability of the mitochondrial membranes on which the enzyme is located. It was assumed for the purpose of this investigation that changes in the catalytic properties of MAO during exposure to different extremal factors have a common mechanism, and accordingly, the Michaelis constant (K_m) or the principal substrate of type A MAO, namely serotonin, and activity of the enzyme in the mitochondria and cytoplasm in hyperoxia, hypoxia, and cold stress were determined.

EXPERIMENTAL METHOD

Adult noninbred albino rats of both sexes weighing 150-180 g, exposed to hyperoxia (0.7 MPa O_2, convulsive state), hypoxic hypoxia (900 m above sea level, 1 h) or kept in a cold chamber at 2°C for 3 days (cold stress) were the test objects. Rats taken from the same group and kept in the animal house at 20-22°C served as the control. Mitochondria were isolated by differential centrifugation [8]. Activity of type A MAO (serotonin as substrate) and the intensity of glucosamine deamination were determined, as described in [5], in brain mitochondria and in the supernatant containing microsomes, lysosomes, and cytosol. On the basis of values of enzyme activity in the presence of different concentrations of serotonin, K_m was calculated graphically by the Lineweaver–Burk method. Serotonin was used in concentrations from $7.5 \cdot 10^{-5}$ to $2.5 \cdot 10^{-3}$ M. Protein was determined by a modified Lowry's method [12]. To study the effect of chlorgyline, the enzyme preparation was preincubated for 15 min at 20°C with the inhibitor in a concentration of 10^{-6} M, in which it has the maximally selective action on type A MAO [14].

EXPERIMENTAL RESULTS

The study of K_m for the serotonin deamination reaction in the mitochondrial fraction of the brain revealed an increase in its value by 179% in hyperoxia, by 118% in hypoxia, and by 129% during cold stress compared with

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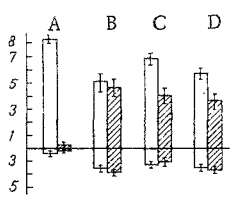

Fig. 1

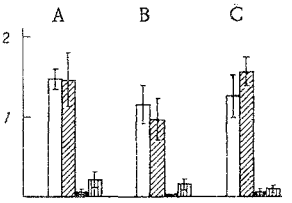

Fig. 2

Fig. 1. Deamination of serotonin (unshaded columns) and glucosamine (shaded columns) in mitochondria (above the ordinate) and cytosol (below the ordinate) under normal conditions (A) and during hyperoxia (B), hypoxia (C), and cold stress (D). Ordinate, intensity of deamination (in nanomoles ammonia nitrogen/mg protein/min).

Fig. 2. Effect of chlorgyline on deamination of serotonin and glucosamine in cytosol in hyperoxia (A), hypoxia (B), and cold stress (C). Ordinate, intensity of deamination (in nanomoles ammonia nitrogen/mg protein/min). Unshaded columns – deamination of serotonin without inhibitor, oblique shading – deamination of glucosamine without inhibitor, black columns – effect of chlorgyline on serotonin deamination, vertical shading – effect of chlorgyline on glucosamine deamination.

the control. K_m was 0.14 ± 0.01 mM in intact animals, 0.39 ± 0.06 mM ($P < 0.001$) in hyperoxia, 0.31 ± 0.045 mM ($P < 0.01$) in hypoxia, and 0.32 ± 0.05 mM ($P < 0.001$) in cold stress. The increase in K_m is evidence of a decrease in affinity of the enzyme and substrate, in agreement with the fall in activity of type A MAO. Activity of type A MAO in the mitochondrial fraction fell by 39% in hyperoxia, by 18% in hypoxia, and by 30% in cold stress. Consequently, correlation was found between the degree of inhibition of MAO and the increase in K_m. The highest value of K_m and the greatest fall in type A MAO activity were found in hyperoxia and the lowest K_m and the smallest fall of activity in hypoxia; cold stress occupied an intermediate position for both parameters.

Inhibition of the enzyme in the mitochondria was accompanied by the appearance of serotonin-deaminase activity in the supernatant (Fig. 1). Activity of type A MAO in this fraction was 4.4 times higher than the control level in hyperoxia, 3.7 times higher in hypoxia, and 3.5 times higher during cold stress. There was a parallel decrease in monoamine-deaminase activity of mitochondrial MAO with a change in substrate specificity of the enzyme, as shown by the appearance of ability to deaminate glucosamine, which is normally not a substrate for MAO. The appearance of glucosamine-deaminase activity was observed in the supernatant also in all the states studied.

To determine whether the appearance of serotonin-deaminase and glucosamine-deaminase activity in the supernatant was in fact connected with MAO, the effect of preincubation with the selective inhibitor of type A MAO, chlorgyline, was investigated. It was shown previously that chlorgyline prevents the potentiation of deamination of glucosamine and other unusual substrates in the mitochondria during hyperoxia, hypoxia, and cold stress, evidence of a change in substrate specificity of type A MAO under these conditions [2, 4, 5]. It will be clear from Fig. 2 that chlorgyline, in a concentration of 10^{-6} M, completely prevents any increase in the intensity of deamination of serotonin and glucosamine in the supernatant. Consequently, the increase in serotonin-deaminase and glucosamine-deaminase activity in this fraction in hyperoxia, hypoxia, and cold stress was due to type A MAO.

The fall in MAO activity in the mitochondria and its appearance in the supernatant (i.e., in the cytosol) are evidence of a change in structure and permeability of the mitochondrial membranes in hyperoxia, hypoxia, and cold stress, and they suggest that disturbance of the connection between MAO and the mitochondrial membranes is one of the universal mechanisms of enzyme inhibition. Enzyme appearing in the cytosol was able to deaminate glucosamine, which is characteristic of type A MAO with modified substrate specificity. Both a change in substrate specificity of MAO induced by partial oxidation of some sulfhydryl groups of the enzyme [1] and, evidently, disturbance of membrane structure were due to lipid peroxides, which accumulate during hyperoxia, hypoxia, and cold stress [6, 9, 10].

The similar modification of the catalytic properties of MAO discovered in various extremal states (hyperoxia, hypoxia, exposure to cold) was thus due to two factors: extrusion of the enzyme into the cytoplasm as a result of disturbance of membrane structures and a change in the molecular properties of MAO, as shown by an increase in K_m and changes in the substrate specificity of the enzyme.

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PYRENE PROBE STUDY OF MEMBRANE LIPIDS OF THE SARCOPLASMIC RETICULUM IN ISOPROTERENOL MYOCARDITIS

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The cardiac rhythm is largely determined by operation of the Ca pump of the myocardial sarcoplasmic reticulum (SR). In turn, in various forms of pathology of the heart, disturbance of the working of this pump and its regulation [1] leads to incompleteness of diastole and, consequently, of systole also. Membrane-bound enzyme function also is known to depend largely on the state of the surrounding lipids, which determines the flowability and microviscosity of the membrane [6].

Accordingly, in the investigation described below, the state of the lipid layer of SR membrane and activity of the Ca,Mg-ATPase of SR of the heart muscle were studied in isoproterenol myocarditis.

EXPERIMENTAL METHOD

Myocarditis was induced in rabbits weighing 1.5-2.0 kg by subcutaneous injection of a 1% aqueous solution of isoproterenol (1 mg/kg body weight). The animals were decapitated 24 h after injection of the drug. Ischemic tissue from the left ventricle was investigated and the same regions of the myocardium of healthy animals served as the control. The microsomal fraction was isolated by the method in [7]. Excimerization of pyrene in SR was

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