EFFECT OF ADRENALIN, NORADRENALIN, DOPAMINE, DOPA, AND PHENYLALANINE ON PEROXIDATION OF LIPIDS IN LIVER MITOCHONDRIAL MEMBRANES

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The effect of catecholamines on chain peroxidation of lipids in the liver mitochondrial membranes in the presence of Fe^{++} ions was studied by a very weak chemiluminescence method. Catecholamines between concentrations of 10^{-6} and 10^{-4} M were shown to inhibit this process. By a mathematical investigation of the kinetics of the process constants of antioxidative activity of the catecholamines were calculated, as follows: for noradrenalin $1.13 \cdot 10^4$ M^{-1}, adrenalin $1.04 \cdot 10^4$ M^{-1}, dopamine $7.6 \cdot 10^3$ M^{-1}, and dopa $5 \cdot 10^3$ M^{-1}. The antioxidative action of the catecholamines was linked with the presence of a free phenol group in their molecule. The mechanism of inhibition consists of interaction between catecholamines and free radicals leading the oxidation chains. It is postulated that the antioxidative action of catecholamines may have an important role in the regulation of the permeability of biological membranes.

KEY WORDS: catecholamines; peroxidation of lipids; membranes; antioxidants; chemical kinetics.

One of the mechanisms regulating the permeability of biological membranes may be peroxidation of unsaturated fatty acids [1, 2, 7]. This process is constantly taking place in the lipid phase of cell membranes. Presumably the action of catecholamines on biological membranes [5] is connected with their effect on peroxidation of lipids.

The action of adrenalin, noradrenalin, dopamine, dopa, and phenylalanine on peroxidation of lipids was studied in a suspension of liver mitochondria in the presence of Fe^{++} ions, which induce this process.

EXPERIMENTAL METHOD

Mitochondria were isolated from rat liver in a medium containing 0.25 M sucrose and 2.5 mM tris-HCl buffer, pH 7.4. Protein was determined by Lowry's method. The residue of mitochondria was diluted in the isolation medium at the rate of 18 mg protein to 1 ml suspension. Chemiluminescence accompanying the chain of peroxidation of lipids in the mitochondrial membranes was measured on an apparatus for recording very weak fluorescence [10]. The chemiluminescence detector was a sensitive FÉU-39A photomultiplier. The signal, led through an ADD-1 automatic differential discriminator, was amplified by a type USh-1 wideband amplifier and recorded on an ÉPP-09 electronic potentiometer. The potential from a VSV-2S high-voltage stabilizer was led to the FÉU-39A photomultiplier. The constant-temperature (37°C) measuring cell contained 8.6 ml of incubation medium (20 mM phosphate buffer in 0.105 M KCl, pH 7.4), 1 ml of mitochondrial suspension, and 0.4 ml of aqueous solution of the test compound. After 4 min (when the temperatures of the suspension and the cell were

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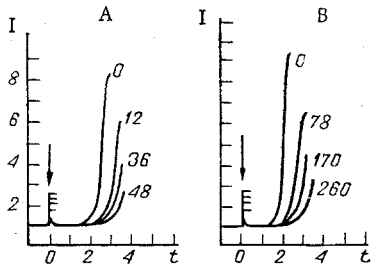

Fig. 1

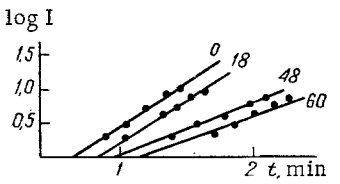

Fig. 2

Fig. 1. Effect of adrenalin (A) and dopamine (B) on chemiluminescence of mitochondria in the presence of Fe^{++} ions: I) intensity of chemiluminescence (in relative units), t) time after addition of $FeSO_4$ (in min), time of addition of Fe^{++} indicated by arrow. Numbers by curves show final concentrations of catecholamines (in μM).

Fig. 2. Log I as a function of time t in initial stage of "slow" flash in the presence of Fe^{++} ions and different concentrations of adrenalin (in μM).

equalized) the dark current and the intrinsic fluorescence of the mitochondria were measured, and 2 min later the suspension was treated with 10 ml of 10 mM $FeSO_4$ solution. Control experiments showed that at the time of addition of the $FeSO_4$ practically all of the test preparation was incorporated into the mitochondrial membranes. The "fast" and "slow" flashes of chemiluminescence were then recorded. The experiment ended with a repeated measuring of the dark current.

Aqueous solutions of adrenalin hydrotartrate, noradrenalin hydrotartrate, dopamine, dopa, and phenylalanine were used in the experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

During investigation of the action of catecholamines and dopa in concentrations of 10^{-6}-10^{-4} M delay in the development of chemiluminescence and a decrease in amplitude of the "fast" and "slow" flashes were observed in all experiments (Fig. 1). This is evidence of inhibition of lipid peroxidation in the mitochondrial membranes. The phenomena observed are similar to those taking place during the action of such antioxidants as α-tocopherol [4], synthetic antioxidants [6], and steroid hormones [3, 9].

To estimate the antioxidative action of catecholamines quantitatively and to attempt to explain the mechanism of this effect the kinetics of the process was studied [3].

The intensity of fluorescence I in the initial stage of the ascending portion of the "slow" flash of chemiluminescence is an exponential function of the time t. Hence, we find that:

$$\lg I = \lg I_0 + 0.868 \cdot \delta \cdot t,$$

where the coefficient δ depends on the concentration of the antioxidant [InH] in accordance with the equation

$$\frac{\delta_0}{\delta} - 1 = A\,[\mathrm{In\,H}], \qquad (1)$$

where A is a constant characterizing the activity of the antioxidant and equal to the reciprocal of the concentration of the substance reducing the value of δ by half.

The linear relationship of log I and t in the initial stage of the "slow" flash and the decrease in the angle of slope α of these lines ($\tan \alpha = 0.868\delta$) in the presence of the antioxidant were demonstrated during a study of the action of α-tocopherol [4] and of steroid hormones [3].

TABLE 1. Antiradical Activity of Catecholamines toward Lipid Peroxidation in Mitochondrial Membrane

Compound	Concentration (C) reducing index δ by half (in M)	Activity (in M^{-1})
Noradrenalin	$8{,}8 \cdot 10^{-5}$	$1{,}13 \cdot 10^{4}$
Adrenalin	$9{,}6 \cdot 10^{-5}$	$1{,}04 \cdot 10^{4}$
Dopamine	$1{,}31 \cdot 10^{-4}$	$7{,}6 \cdot 10^{3}$
Dopa	$2{,}06 \cdot 10^{-4}$	$5{,}0 \cdot 10^{3}$
Phenylalanine	—	Inactive

The results shown in Fig. 2 were obtained by the investigation of a suspension of mitochondria in the presence of adrenalin. Under the influence of this substance the angle of slope of the straight lines expressing δ as a function of t was reduced. Similar results were obtained for noradrenalin, dopaamine, and dopa. The experimental results show that catecholamines are antioxidants and that their antioxidative action is based on the reaction of the inhibitor InH with free RO_2 radicals leading the oxidation chain [2]:

$$InH + RO_2 \cdot \xrightarrow{k_7} In + ROOH.$$

The antioxidative activity (A) of the catecholamines was calculated by equation (1). The concentration of the preparation reducing the value of δ by half also was found (Table 1).

It can be concluded from these results that compounds with a free phenol group in their chemical structure possess antioxidative activity. For instance, phenylalanine with no OH-group in the benzene ring was ineffective, whereas catecholamines and dopa inhibited peroxidation of lipids. An action similar to that of catecholamines is shown, in particular, by steroid hormones, whose activity is also connected with the presence of a free phenol group [9]. Compared with steroid hormones, the strength of whose action may differ by as much as 100 times, the activity of the catecholamines differs by not more than twice.

The antioxidative activities of the catecholamines and steroids are fully comparable and are values of the same order of magnitude.

It can be postulated that catecholamines lead to inhibition of peroxidation of lipids not only in the mitochondrial membranes of the liver but also in other membranes. The possibility cannot be ruled out that catecholamines, by interacting with biological membranes, may affect their own passage through them. The study of these phenomena is particulary important to the understanding of the function of the adrenergic synapse and the principles of its pharmacological regulation. The process of lipid peroxidation controls the permeability of several different membranes [2, 11-13]; consequently, inhibition of peroxidation by catecholamines may alter the permeability of biological membranes for ions and metabolites, and also the activity of enzymes which, in turn, regulate permeability processes. There are data in the literature, in particular, to the effect that catecholamines lower the redox potential of the myocardium in the intact heart, increase pH and pNa, increase the duration of local monophasic action potentials, and reduce the velocity of the third repolarization phase [8].

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