EFFECT OF TRYPTOPHAN, 5-HYDROXYTRYPTOPHAN, SEROTONIN, HISTIDINE, AND HISTAMINE ON PEROXIDATION OF LIPIDS IN LIVER MITOCHONDRIAL MEMBRANES

P. V. Sergeev, R. D. Seifulla,
V. G. Dunaev, and Yu. M. Rudnev

UDC 612.352.2-06:612.822.2

The effect of tryptophan, 5-hydroxytryptophan, serotonin, histidine, and histamine on peroxidation of lipids in rat liver mitochondrial membranes in the presence of Fe^{++} ions which induce this process was studied by recording very weak chemiluminescence. 5-Hydroxytryptophan and serotonin in concentrations of 10^{-5}-10^{-4} M (protein concentration 1.8 mg/ml mitochondrial suspension) inhibit this process. By studying the kinetics of the initial part of the ascending branch of the "slow" burst constants of antioxidative activity were calculated: For 5-hydroxytryptophan and serotonin these were $2.2 \cdot 10^3$ and $9.8 \cdot 10^3$ M^{-1} respectively. The antioxidant action is linked with the presence of a phenol group in the molecule of the compound tested. It is postulated that the action of 5-hydroxytryptophan and serotonin on peroxidation of lipids in membranes, besides their effect on other membrane processes, is also an important factor in the regulation of permeability of biological membranes.

KEY WORDS: peroxidation of lipids; membranes; biogenic amines; mitochondria; antioxidants.

The peroxidation of lipids is a universal free-radical process taking place at very slow velocity in the lipid phase of biological membranes [1]. This process is controlled by several enzyme systems. Different compounds can modify the peroxidation of lipids, but they mainly inhibit it. For instance, α-tocopherol [3] is an inhibitor of this process. Depending on their chemical structures steroid hormones also substantially inhibit the peroxidation of membrane lipids [2], and the phenol group is responsible for the antioxidative activity of the steroid molecule [5]. Catecholamines also have antioxidative activity in relation to lipid peroxidation [6].

The object of this investigation was to study the effect of tryptophan 5-hydroxytryptophan, serotonin, histidine, and histamine on the peroxidation of lipids in a liver mitochondrial suspension in the presence of Fe^{++} ions, which catalyze this process.

EXPERIMENTAL METHOD

Mitochondria were isolated from rat livers in medium containing 0.25 M sucrose and 2.5 mM tryptophan, HCl, 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 process of chain peroxidation of lipids in the mitochondrial membranes was measured on an apparatus for recording very weak luminescence [7]. The chemiluminescence detector was a sensitive FEU-39A photomultiplier. The signal was led through an ADD-1 automatic differential discriminator, amplified by a USh-1 wide-band amplifier, integrated, and recorded on an ÉPP-09 electronic potentiometer. The voltage on the

Department of Molecular Pharmacology and Radiobiology, Medicobiological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 169-171, February, 1976. Original article submitted February 25, 1975.

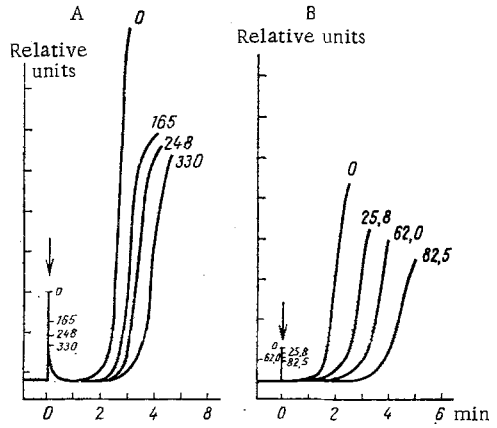

Fig. 1

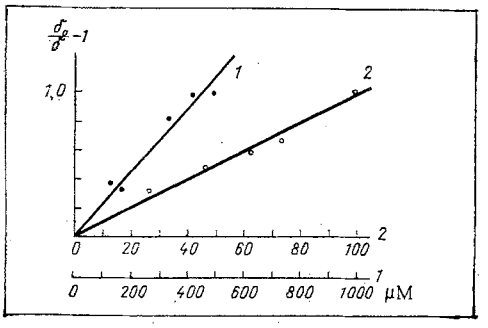

Fig. 2

Fig. 1. Effect of 5-hydroxytryptophan (A) and serotonin (B) on chemiluminescence of mitochondria in the presence of Fe^{++} ions. Abscissa, time (t) after addition of $FeSO_4$ (in min); ordinate, intensity of chemiluminescence (I, in relative units). Arrow indicates time of adding Fe^{++}. Numbers by curves show final concentrations of compounds (in μM).

Fig. 2. δ_0/δ as a function of concentration of 5-hydroxytryptophan (1) and serotonin (2).

FEU-39A was obtained from a VSV-2S high-voltage stabilizer. The constant-temperature (37°C) measuring cell contained 8.6 ml incubation medium (20 mM phosphate buffer in 0.105 M KCl, pH 7.4), 1 ml mitochondrial suspension containing 18 mg protein, and 0.4 ml of an aqueous solution of the test compound. The dark current and intrinsic luminescence of the mitochondria were measured 4 min after equalization of the temperature of the suspension and thermostat, and 1 ml of a 10 mM solution of $FeSO_4$ was added to the suspension 2 min later. Control experiments showed that by this time virtually all the test compounds had been taken up by the mitochondrial membranes. The "fast" and "slow" bursts of chemiluminescence were then recorded. The experiment ended with a repeated measurement of the dark current.

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that 5-hydroxytryptophan and serotonin, within a concentration range of 10^{-6}–10^{-4} M, increased the latent period of chemiluminescence and reduced the amplitude of the "fast" and "slow" bursts (Fig. 1). These observations indicate inhibition of lipid peroxidation in the mitochondrial membranes.

To estimate the antioxidant action of 5-hydroxytryptophan and serotonin quantitatively and to discover the mechanism of this effect, the kinetics of the process was studied [1]. The antioxidative activity (A) of antioxidants is described by the equation:

$$\frac{\delta_0}{\delta} - 1 = A \cdot [InH],$$

where InH is the antioxidant concentration and δ an index of the degree of the initial stage of the "slow" burst.

It will be clear from Fig. 2 that this equation also holds good for biogenic amines. Besides the antioxidative activity A, the concentration of the compound reducing the value of δ by half also was found (Table 1).

The investigations show that 5-hydroxytryptophan and serotonin are antioxidants and that their antioxidative activity is based on a reaction between the inhibitor InH and free RO_2 radicals leading the oxidation chain [1].

Comparison of the results shows that compounds with a free phenol group in their chemical structure possess antioxidative action. For instance, 5-hydroxytryptophan and serotonin inhibited peroxidation of lipids in the mitochondrial membranes, whereas tryptophan, histidine, and histamine, with no OH group attached to their benzene ring, were inactive. The phenomena observed are analogous to those taking place under the influence of antioxidants such as α-tocopherol [3], synthetic antioxidants [4], steroid hormones [5], and catecholamines [6]. Comparison of the indices of antioxidative activity of the series of

TABLE 1. Antioxidative Activity of Biogenic Amines Relative to Peroxidation of Lipids in Mitochondrial Membranes

Compound	Concentration reducing δ by half (in M)	A (in M^{-1})
Tryptophan	—	Inactive
5-Hydroxytryptophan	$4{,}6 \cdot 10^{-4}$	$2{,}2 \cdot 10^{3}$
Serotonin	$1{,}02 \cdot 10^{-4}$	$9{,}8 \cdot 10^{3}$
Histidine	—	Inactive
Histamine	—	

compounds tested, steroid hormones [5], and catecholamines [6] showed that they are of the same order of magnitude.

The structural basis of all biological membranes is a bimolecular layer of phospholipids. Peroxidation of membrane lipids is a universal process characteristic of membranes in general and it is one of the mechanisms controlling their permeability [1]. That is probably why biogenic amines, by inhibiting peroxidation in the lipid phase of the mitochondrial membranes used in the present experiments as a general model of biomembranes, could have a similar effect also in other membranes; they could ultimately modify their permeability. If this is so, the antioxidative action of biogenic amines could be one aspect of their broad spectrum of biological activity.

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