EFFECT OF THE ANTIOXIDANT EMOXYPINE ON LIPID METABOLISM IN THE LUNGS DURING DEVELOPMENT OF PULMONARY EDEMA

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Preliminary administration of antioxidants lowered the resistance of albino rats only to those factors inducing pulmonary edema which caused intensification of lipid peroxidation (LPO) in that organ [8]. There is reason to suppose that the more marked development of pulmonary edema (PE) against the background of emoxypine* is in fact linked with its antioxidant properties [10]. Administration of antioxidants in excess is known to lead to activation of LPO in the tissues [13]. It therefore seemed important to study the effect of emoxypine, a water-soluble antioxidant, on lipid metabolism in the lungs during the development of pulmonary edema and to determine whether its intensification against the background of this preparation is in fact combined with inhibition of LPO.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male albino rats. PE was induced by intravenous injection of pituitrin (10 U/kg), for the edema-inducing action of emoxypinehas been observed most clearly on this model [10]. Emoxypine (100 mg/kg) was injected intraperitoneally daily for 4 days, the last injection being given 1 h before induction of edema. The rats were killed 40 min after injection of pituitrin by decapitation on a guillotine. The right lung was used to assess the intensity of PE on the basis of the value of the lung coefficient (LC), the dry residue of the lungs (DR), the volume of edema fluid (EF), and the gain in blood volume in the lungs (BV), expressed in g/kg body weight [15]. The left lung was used to obtain a lipid extract by treating 1 ml of homogenate (300 mg tissue in 1 ml Tris-HCl, pH 7.4, with addition of 1 mM EDTA) with a chloroform-methanol mixture [1]. To avoid activation of LPO during extraction of the lipids, the antioxidant 2,6-di-tert-butylphenol was added to the extract, and nitrogen was bubbled through the samples. After evaporation of the portions of lipid extract in vacuo, the following parameters were determined in them: concentration of total lipids by Bragdon's method, total cholesterol by the Liebermann—Burchard method [9], total phospholipids with the aid of Malachite Green [14], nonesterified fatty acids (NEFA) by a photometric method using diethyldithiocarbamate, and the content of LPO products by UV-spectroscopy (conjugated dienes, ketodienes) in a mixture of heptane and isopropyl alcohol, 1:1 [5]. The residue of the homogenate was used to determine dry mass contained in 1 ml of homogenate.

The lipid content was expressed in g/kg dry lung tissue, and the content of cholesterol, phospholipids, and MEFA in mmoles/kg dry tissue. The content of conjugated dienes (CD) and ketodienes (KD) was calculated in optical density units/mg lipids.

To confirm the results, in some experiments the powerful LPO inducer butylhydroperoxide, in physiological saline (dilution $1 \cdot 10^{-5}$ M) in a volume of 5 ml/kg, or physiological saline alone, was injected intratracheally in a series of

*2-Ethyl-6-methyl-3-oxypyridine.

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TABLE 1. Effect of Emoxypine on Parameters of Lipid Metabolism in Lungs of Rats with Pituitrin-Induced Pulmonary Edema (M ± m)

Parameter	Control	Emoxypine + pituitrin	Pituitrin
Parameters of lipid metabolism	129,8±3,74	147,1±10,5	105,5±7,6*
Total lipids, g/kg	56,14±2,68*	36,0±3,6	43,54±2,70
Total cholesterol, mmoles/kg	277,9±11,5	287,6±10,1	203,5±17,6*
Total phospholipids, mmoles/kg	50,2±3,5	45,56±3,5	39,8±3,4
NEFA, mmoles/kg	0,45±0,04*	0,25±0,03	0,71±0,03*
CD, optical density units/mg lipids	0,24±0,02*	0,13±0,02	0,35±0,02*
Ketodienes, optical density units/mg lipids			
Parameters of intensity of edema:			
Lung coefficient, g/kg	3,92±0,22*	9,61±0,48	9,05±0,85
Dry residue, g/kg	21,09±0,40*	12,02±0,65	14,32±0,72*
Edema fluid, g/kg	0,00±0,16*	4,12±0,32	3,00±0,44
Degree of filling of blood vessels, g/kg	0,00±0,43*	1,56±0,62	2,13±0,51

Legend. *$p < 0.05$ denotes significant difference from group with emoxypine.

experiments. These animals were killed 2 h after injection of the solutions, and the intensity of PE was subsequently assessed. Ten animals were used in each series.

EXPERIMENTAL RESULTS

Injection of pituitrin (Table 1) led to the development of marked PE ($p < 0.001$) and to an increase in the pulmonary blood volume ($p < 0.01$). The development of edema was combined with a decrease in the content of total lipids in the lungs by 18.7% ($p < 0.01$), total cholesterol by 22.4% ($p < 0.01$), and total phospholipids by 26.8% ($p < 0.01$) and of NEFA by 20.7% ($p < 0.01$), with a simultaneous increase in NEFA in the blood serum by 64.2% ($p < 0.05$). The content of CD in the lungs increased by 57.8% ($p < 0.02$) and that of KD by 45.8% ($p < 0.001$), evidence of activation of LPO. Preliminary injection of emoxypine prevented the fall of the levels of total lipids ($p < 0.01$), phospholipids ($p < 0.001$), and MEFA in the lungs in response to injection of pituitrin, but promoted a further decrease in the cholesterol content in the lungs by 17.3% (by 36% compared with intact animals, $p < 0.001$, see Table 1). This may perhaps be connected with the fact that injection of exogenous antioxidants is accompanied by a decrease in the intrinsic antioxidative activity of the tissues [13]. Cholesterol is known to be a natural inhibitor of LPO, and a decrease in its content leads to potentiation of radical formation [2]. Intensification of LPO in the lungs due to injection of pituitrin is probably also connected with a fall in the cholesterol concentration in the lungs. Preliminary injection of emoxypine blocked this process. For instance, the content of CD fell by comparison with the control group (pituitrin alone) by 64.8% ($p < 0.001$), and the KD content fell by 63% ($p < 0.001$). The level of these products in the lungs, moreover, was even lower than their levels in intact rats, by 44.4% ($p < 0.05$) and 46% ($p < 0.01$) respectively. The volume of BF in the right lung increased at the same time by 37.3%, whereas the dry residue fell by 16.1% ($p < 0.05$), without any increase in the pulmonary blood volume (Table 1). Thus the membrane-stabilizing action of emoxypine on the lungs with depression of LPO in them was accompanied by the development of more marked PE. This is in agreement with our data showing that intravenous injection of olive oil with an increased content of CD caused the development of a less marked degree of PE, whereas injection of a 10% solution of tocopherol in olive oil, on the contrary, was accompanied by an additional increase in the volume of EF in the lungs by 200% compared with its level after injection of olive oil alone [7]. Very probably the presence of positive correlation between the degree of PE and the cholesterol content in the lungs [7] also is linked with its antioxidant properties. The results agree also with data showing that 3-oxypyridine derivatives, and emoxypine in particular, possess marked ability to inhibit enzymic and nonenzymic LPO in different organs and tissues, and the ability to stabilize the phospholipid, fatty acid, and protein composition of the membranes during initiation of LPO [4], and ability to enhance the pharmacologic effect of several preparations [12].

Our data are in agreement with the conclusion drawn by Kryzhanovskii [6] that changes arising in membranes in connection with potentiation of LPO can cause a disturbance of reactivity of the receptors contained in them and their ability to bind biologically active substances or pharmacologic preparations. This may be the cause of tolerance to the action of humoral factors and to the ineffectiveness of these preparations. The use of antioxidants, capable of normalizing

receptor reactivity leads to a decrease in tolerance and an increase in the effectiveness of pharmacotherapy. Clearly these properties are highly conspicuous in emoxypine. The facts described above do not rule out the possibility of damage to the lung membrane by active forms of oxygen [3]. This effect, however, is not necessarily connected with activation of LPO. In experiments on isolated rat lungs, for instance, development of PE during perfusion of the lungs with oxygenated solution was shown to be not connected with activation of LPO in the lungs [11]. According to our own data, intratracheal injection of butylhydroperoxide was not accompanied by additional accumulation of EF in the lungs compared with injection of physiological saline (EF 1.34 ± 0.35 compared with 0.90 ± 0.27, $p > 0.1$). Thus inhibition of LPO processes in the lungs during induction of pulmonary edema, inducing activation of LPO, may increase the intensity of PE. Emoxypine, by preventing the accumulation of hydrolyzed residues of phospholipids and free fatty acids, and by suppressing LPO, potentiates the effect of receptor–ligand interaction (or increases the intensity of the signal from the receptor to the intracellular effector systems), an effect manifested as a stronger response to stimulating factors.

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