EFFECT OF LIPID PEROXIDATION PRODUCTS ON VASCULAR TONE

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The causes and molecular mechanisms of postischemic hemodynamic disturbances have not yet been discovered although evidence of the possible role of cateholamines and tissue mediators [9, 13] and also of metabolites of protein nature [8, 12] in their genesis has been obtained.

We know that ischemia of various organs and their reperfusion are accompanied by intensification of lipid peroxidation (LPO) in membranes and the accumulation of primary (hydroperoxides of phospholipids) and secondary (aldehydes, ketones) products in them [1, 2], as well as by an increase in the concentration of LPO products in blood flowing from the ischemized organ [5]; the degree of intensification of LPO correlates, moreover, with the period of ischemia, the sensitivity of the organ to it, and the severity of the hemodynamic disorders arising in the organ [4]. Accordingly, the writers postulated previously a possible role of substances of lipid peroxide nature in the mechanism of the postischemic vasoconstrictor reaction [3]. In fact several products of lipid nature — lysophospholipids, protagalndins E_2 and $F_{2\alpha}$, and also endoperoxides and hydroperoxides of protaglandins — possess vasoconstrictor properties [14, 18]. Free fatty acids (FA), whose content rises sharply in ischemized tissue and blood, evoke a vasoconstrictor [13] or, according to data of other workers [10, 15], a vasodilator reaction. The vasoactive effect of hydroperoxides of phospholipids and of secondary products formed during LPO has not been investigated.

The study of the effect of LPO products on vascular tone was the aim of the investigation now described.

EXPERIMENTAL METHODS

Experiments were carried out on a model of perfusion of the vessels of the isolated rabbit ear by a modified method in [7]. The perfusion fluid consisted of Ringer's solution, which was injected through a cannula into the posterior auricular artery under a pressure of 100 mm water at a temperature of 20°C. The volume velocity of perfusion was recorded by means of an SB-1M-100 electromechanical counter with simultaneous automatic recording. The readings were taken before injection of LPO products (initial value) and during the 10 min after injection of the test substances. The volume velocity during reperfusion was expressed as a percentage of the initial value (a change in the values by not less than 10% of the initial reading was taken as a decrease or increase). LPO products were obtained by oxidation of phosphatidylethanolamine (PEA) by lipo-oxygenase at 4°C with a continuous supply of oxygen, in Tris-HCl (pH 7.8), followed by isolation of PEA hydroperoxides on plates covered with a thin layer of silica gel, in a system of organic solvents: chloroform—methanol—28% NH_4OH (13:7:1). PEA was obtained from egg yolk by column chromatography [16]. The method developed allowed preparations enriched with PEA hydroperoxides by 25-30% to be obtained. Secondary products of PEA were obtained by incubating PEA hydroperoxides for 48 h at 37°C. Hydroperoxides of FA were obtained from arachidonic acid (commercial preparation) by a modified method [17]. Lipids were suspended in 1.0 ml of Ringer's solution and injected into an artery of the rabbit's ear from a syringe. Altogether 126 experiments were carried out on gray rabbits. The results were subjected to statistical analysis by means of Pearson's χ^2 test.

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TABLE 1. Changes in Volume Velocity of Perfusion of Rabbit Ear Vessels under the Influence of Lipids

Preparation	Dose, μmoles	Number of experiments	Effect, number of cases		
			decrease	increase	no change
PEA	0,01	8	1 (12)	5 (63)	2 (25)
	0,1	9	3 (33)	3 (33)	3 (34)
	1,0	10	2 (40)	4 (40)	2 (20)
	10,0	10	2 (20)	7 (70)	1 (10)
Hydroperoxides of PEA	0,001	5	2 (40)	1 (20)	2 (40)
	0,01	21	15 (71)	1 (5)	5 (24)
	0,1	19	10 (53)	7(37)	2 (10)
	1,0	8	1 (12)	3 (38)	4 (50)
Secondary products of PEA, FA	0,02—0,2	10	6 (60)	2 (20)	2 (20)
	1,0	13	2 (15)	4 (31)	7 (54)
Hydroperoxides of FA	0,2—0,5	13	5 (39)	5 (39)	3 (22)

Legend. Figures in parentheses are percentages of total number of experiments.

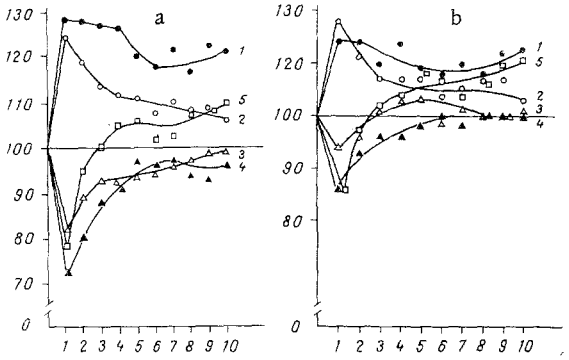

Fig. 1. Time course of volume velocity of perfusion of rabbit ear vessels in response to injection of PEA and its oxidation products. a) Mean data for all experiments; b) mean data for experiments with vasodilator (PEA) or vasoconstrictor (hydroperoxides, secondary products of PEA) effects. 1) PEA (0.01 μmole); 2) PEA (10 μmoles); 3) PEA hydroperoxides (0.01 μmole); 4) PEA hydroperoxides (0.1 μmole); 5) secondary products of PEA (0.02-0.2 μmole). Abscissa, time of reperfusion (in min); ordinate, volume velocity of perfusion (in % of initial).

EXPERIMENTAL RESULTS

Injection of unmodified PEA into the vessels of the ear was accompanied in 19 of 37 cases (51%) by an increase in the volume velocity of perfusion, i.e., by a decrease in vascular tone (Table 1). Differences between the number of cases when the volume velocity of perfusion was increased and the number when it was unchanged or reduced were significant ($P < 0.01$). The effect was most marked when PEA was injected in doses of 0.01 and 10 μmoles. An increase in the volume velocity of perfusion when these doses were used was observed in 63 and 70% of cases, respectively. Injection of PEA in doses of 0.1 and 1.0 μmole had no definite consistent effect, possibly on account of differences in the direction of action of the preparation in different doses.

Unlike PEA, injection of its hydroperoxides reduced the volume velocity of perfusion in 28 of 55 cases (53%). The vasoconstrictor effect was most marked when PEA hydroperoxides were given within a narrow dose range of from 0.01 to 0.1 mole (a decrease in the volume velocity of perfusion in 71 and 53% of cases, respectively; $P < 0.001$). Injection of PEA hydroperoxides in lower (0.001 μmole) and higher doses (1.0 μmole) had no clear consistent effect. The decrease in the vasoconstrictor effect of PEA hydroperoxides when the dose was

increased to 1.0 μmole was evidently due either to the possibility that different doses of the preparation had opposite effects, which is a feature of many biologically active substances, or to a simultaneous increase in the content of unmodified lipid in the preparation, as well as PEA hydroperoxides, and which, as was shown above, has a vasodilator effect.

Secondary products of PEA in 60% of experiments caused a decrease in the volume velocity of perfusion and this effect was obtained with all doses used.

The vasodilator action of PEA or the vasoconstrictor action of its oxidation products was maximal at the first minute of reperfusion of the auricular vessels with Ringer's solution (Fig. 1). This was clearly manifested both when the mean volume velocity of perfusion was calculated from the results of the whole series of experiments undertaken with the dose of the preparation used (Fig. 1a) and when the mean index was calculated from the results of only those experiments in which there was a marked vasodilator (PEA) or vasoconstrictor (hydroperoxides, secondary products of PEA) effect (Fig. 1b). The degree of maximal increase in the volume velocity of perfusion in experiments with a vasodilator effect of PEA averaged 124-128%, and the degree of maximal decrease in the experiments with a vasoconstrictor effect of PEA oxidation products averaged 72-82% of the initial level (Fig. 1b). However, the vasodilator effect of PEA continued for 5-10 min of reperfusion, i.e., it was stable in character, whereas the vasoconstrictor effect of hydroperoxides and secondary products of PEA was of shorter duration, and the indices had returned almost to their initial levels after only 3-4 min of reperfusion.

Considering the particular features of the experimental model used — a single injection of the preparation followed by prolonged reperfusion of the vessels with Ringer's solution — differences in the duration of the effects of unmodified and oxidized phospholipids can be attributed to the possession of hydrophilic properties by hydroperoxides and, in particular, by secondary products of PEA, and their possible flushing out of the blood vessels by perfuison fluid. The probability of rapid decomposition of PEA hydroperoxides, by analogy with unstable hydroperoxide intermediates, formed during prostaglandin synthesis [12], likewise cannot be ruled out.

Injection of FA and their hydroperoxides into the blood vessels in the doses used had no clear and consistent action on the tone of the auricular vessels; this result differs from data in the literature [10, 13, 14, 15] and is evidently due to differences in the FA used, the methods of obtaining their derivatives, and the method of evaluation of the action of the preparations of different experimental models.

The results are evidence that primary and secondary peroxidation products of phospholipids, by contrast with unmodified phospholipids, in the doses used have a vasoconstrictor action, confirming the hypothesis that LPO products have a possible role in local and systemic postischemic hemodynamic disturbances, and they establish the need for the development of methods of antioxidant protection for their prevention. The modifying action of LPO products on vascular tone may be realized both by their effect on receptors and by their direct action on the structure and barrier properties of cellular and subcellular membranes responsible for transport of Ca^{2+}, on whose concentration in the sarcoplasm the processes of contraction and relaxation of the muscle fiber depend. Support for the membrane mechanism of the vasoactive action of LPO products is given by the authors' previous observations showing that accumulation of LPO products during ischemia of the limb muscles correlates with a reduction in the Ca^{2+}-accumulating capacity of membranes of the sarcoplasmic reticulum [2], and the addition of exogenous PEA hydroperoxides to fragments of membranes of the sarcoplasmic reticulum of limb muscles *in vitro* leads to a decrease in the Ca^{2+}/ATP ratio [6].

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EFFECT OF VERAPAMIL AND MANGANESE IONS ON ACETYLCHOLINE SENSITIVITY OF THE INTACT AND DENERVATED FROG MUSCLE FIBER MEMBRANE

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One result of interaction between acetylcholine (ACh) and its receptor (AChR) in the muscle fiber is a transient change in ionic permeability of the muscle membrane, followed by a depolarization step of membrane potential (MP). The magnitude of the depolarization response of the denervated rat muscle fiber membrane to microapplication of ACh is reduced by verapamil and its derivative, compound D-600 [3], which can block the inward calcium current in objects such as the cardiomyocyte membrane [9], the molluscan neuron soma [10], or the squid giant axon [5]. Consequently, it can be postulated that the decrease in the ACh potential takes place because of absence of the contribution of Ca^{2+} to its amplitude. However, complete replacement of Ca^{2+} in the solution by their antagonists, Mn^{2+} ions [2], has no significant effect on the value of the ACh potential [3]. All this suggests that the action of verapamil and D-600 on ACh sensitivity of the denervated rat muscle fiber membrane cannot be reduced simply to loss of the contribution of Ca^{2+} to the amplitude of the ACh potential.

Extrasynaptic AChR denervated frog muscle fibers are known to differ from synaptic AChR of the innervated end plate [7].

It was accordingly considered interesting to compare the effect of verapamil on ACh sensitivity of the membrane of denervated and intact frog muscle fibers. The investigation described below was undertaken for this purpose.

EXPERIMENTAL METHODS

Experiments were carried out on the frog sartorius muscle in winter, using the ordinary microelectrode technique. The muscel was denervated under ether anesthesia by extirpation

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