coincides with the sequence of stages of keratitis, evidence that processes of retrograde transport and the functional state of the tissue are interconnected.

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PREVENTION OF CATECHOLAMINE-INDUCED LESIONS OF THE ENDOTHELIUM OF THE RABBIT AORTA BY ALPHA- AND BETA-ADRENORECEPTOR ANTAGONISTS AND LITHIUM HYDROXYBUTYRATE

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Injury to the endothelium and the associated increased permeability of the intima of arteries play a leading role in the initial stages of atherogenesis [7]. Catecholamines, the principal mediator of stress, cause damage to the endothelium of the perfused rabbit aorta and increase its permeability for low-density lipoproteins [4]. Experimental lesions of the myocardium induced by catecholamines and isoproterenol, starting with pycnosis of the cardiomyocyte nuclei and ending with myocytolysis, are known [5, 8]. Lesions of the cardiomyocytes, but not of endothelial cells, have been found in investigations of the perfused heart [8]. Beta-adrenoreceptor antagonists can prevent catecholamine-induced damage to cardiomyocytes [5]. Lithium salts, which inhibit adenylate cyclase [6], like beta-adrenoreceptors, prevent the development of stress-induced myocardial lesions [3].

The authors have studied the action of lithium hydroxybutyrate on endothelial lesions induced by catecholamines in the perfused rabbit aorta and have compared the effects of lithium hydroxybutyrate with those of adrenoreceptors antagonists.

EXPERIMENTAL METHOD

Experiments were carried out on 47 male chinchilla rabbits weighing 3-4 kg. Under pentobarbital anesthesia with artificial ventilation of the lungs the thoracic and abdominal portions of the aorta were mobilized, all lateral branches were ligated, and the aorta was divided into four segments of equal length, which were perfused simultaneously. Lung segments served as the control; the rest were perfused with the test substances in different concentration. Perfusion was carried out under standard conditions [1]. The perfusion fluid was medium 199

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with 10% delipidized calf serum. The vessels were treated for scanning electron microscopy by the method described previously [2]. Endothelial cells were demonstrated by impregnation with silver. The specimens were studied with the PSEM-500 scanning electron microscope with a magnification of 320-640. Endothelial lesions were counted on the microscope screen. In each segment 30 random fields with an area of 0.11 mm^2 were analyzed under a magnification of 320. Lesions in the endothelium were assessed by means of three parameters: the index of damage to the endothelial cells (the total number of argyrophilic and desquamated cells in 1 mm^2), the index of injury to intercellular boundaries (the number of cells with injured intercellular boundaries, including craters, stomata, stigmata, and widened intercellular boundaries, per 1 mm^2), and the area of endothelial edema.

The adrenalin and noradrenalin (NA) were obtained from Sigma, USA, phentolamine, propranolol, and oxprenolol were from Ciba-Geigy, Switzerland), and the connecting tubes and cannulas for perfusion were obtained from LKB, Sweden.

EXPERIMENTAL RESULTS

Perfusion of the rabbit aorta for 1 h with adrenalin in a concentration of 10 μM caused changes in the endothelium of the aorta in the form of injury to the endothelial cells, defects of intercellular junctions, and areas of edema in the endothelium (Table 1) [4]. The addition of 10 μM propranolol (a selective beta-adrenoreceptor antagonist) to the perfusion fluid prevented the formation of morphological defects induced by 10 μM adrenalin (Table 1). On perfusion of the rabbit aorta simultaneously with adrenalin (10 μM) and lithium hydroxybutyrate (10 μM) the number of damaged cells was reduced by 1.8 times, the number of cells with damaged intercellular boundaries by 1.9 times, and the area of edema by 8.5 times.

Under the chosen experimental conditions only high concentrations (10 μM) of catecholamines caused significant morphological injury to the endothelium during perfusion for 60 min. On perfusion of the rabbit aorta with adrenalin and NA simultaneously, the final concentration of both catecholamines, causing similar morphological injuries, was reduced by 133 times to 0.075 μM (Table 2). The dose–effect curve for combined administration of adrenalin and NA on the morphological structure of the endothelium is illustrated in Fig. 1. Whereas the minimal catecholamine concentration at which damage to the endothelium occurred was 0.075 μM, if the concentration was increased to 0.1 μM there was a sharp increase in the number of cells with damaged intercellular boundaries (from 123 ± 40 cells/mm^2 at 0.075 μM to 330 ± 61 cells/mm^2 at 0.1 μM). A further increase in concentration of adrenalin and NA led to an increase in the number of damaged cells, but to a decrease in the number of cells with damaged intercellular boundaries (Fig. 1). The reason may perhaps be that initial damage to the intercellular junctions led to damage to the endotheliocytes. Edema of the endothelium first appeared in response to adrenalin and NA in a concentration of 0.1 μM, and it occupied 1.7 ± 0.6% of the surface examined. With an increase in the total catecholamine concentration to 1, 10, and 100 μM, the area of the luminal surface occupied by edema increased to 1.0 ± 0.06, 3.5 ± 1.0, and 10.0 ± 5.1%, respectively.

TABLE 1. Action of Lithium Hydroxybutyrate and Propranolol on Damaging Effects of Adrenalin

Perfusion fluid	Number of damaged cells in 1 mm^2	Number of cells with injured boundaries in 1 mm^2	Area of edema, %
Control	2.3±0.5	58±10	—
Lithium hydroxybutyrate (10 mM)	5.9±0.9	55±18	—
Adrenalin (10 mM)	22±3*	193±20*	1.7±0.9*
Adrenalin + lithium hydroxybutyrate (10 mM)	12±3*	103±12*	0.2±0.1
Adrenalin + propranolol (10 mM)	3.1±0.6	68±10	—

Legend. Here and in Table 3, *$p < 0.05$ compared with control. Significance of differences estimated by Student's t-test.

TABLE 2. Minimal Catecholamine Concentrations at Which Morphological Lesions Were Found in the Endothelium

Perfusion fluid	Concentration, μm	Number of damaged cells in 1 mm^2	Number of cells with injured boundaries in 1 mm^2
Adrenalin	10	5.9±0.9	193±20
NA	10	6.1±1.0	145±18
Adrenalin + NA	0.075	21±8	123±40

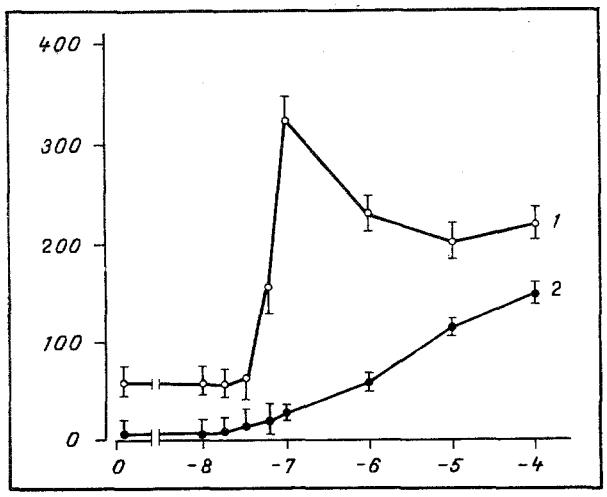

Fig. 1. Dependence of degree of morphological damage to endothelium on adrenalin and NA concentrations in perfusion fluid. Abscissa, adrenalin and NA concentration (in log M); ordinate, number of endotheliocytes in 1 cm^2. 1) index of injury to intercellular boundaries; 2) index of injury to endothelial cells. Perfusion with medium 199 for 1 h at 37°C and with hydrostatic pressure of 100 mm Hg. Each point is the result of at least 5 experiments.

TABLE 3. Prevention of Catecholamine-Induced Lesions by Alpha- and Beta-Adrenoreceptors Antagonists and by Lithium Hydroxybutyrate

Perfusion fluid	Number of damaged cells in 1 mm^2	Number of cells with injured boundaries in mm^2	Area of edema, %
Control	2.3±0.5	58±20	—
Adrenalin + NA (0.1 μM)	22±8*	330±130*	1.7±0.6*
Adrenalin + NA + propranolol (0.1 μM)	2.2±0.9	52±18	—
Adrenalin + NA + phentolamine (10 μM)	5.9±2.6	79±21	—
Adrenalin + NA (10 μM) + lithium hydroxybutyrate (10 μM)	4.2±1.0	63±14	—

Data showing the effects of the alpha-adrenoreceptor antagonist phentolamine, the beta-adrenoreceptor antagonist propranolol, and lithium hydroxybutyrate on the endothelium of the rabbit aorta, damaged as the result of the action of adrenalin and NA (0.1 μM), are given in Table 3. It will be seen that propranolol, phentolamine, and lithium hydroxybutyrate protected the endothelium against the harmful action of catecholamines. The index of injury to the endothelial cells and of the intercellular boundaries was no higher than in the control, nor did edema develop in the endothelium.

Thus lithium hydroxybutyrate (10 μM) partially prevents injury to the endothelium of the rabbit aorta induced by adrenalin (10 μM). The adrenoreceptor antagonists phentolamine and propranolol, in a dose of 10 μM, completely protected the endothelium against the harmful action of 10 μM adrenalin, in agreement with the results of a previous study [4]. Lesions induced in the endothelium by the simultaneous action of 0.1 μM adrenalin and NA were abolished equally effecitvely by alpha- and beta-adrenoreceptor antagonists (phentolamine and propranolol) and by lithium hydroxybutyrate.

Catecholamine-induced injury to the endothelium may perhaps be linked with simultaneous activation of both alpha- and beta-adrenoreceptors. Selective adrenoreceptor antagonists block simultaneous activation of receptors by catecholamines, thus preventing injury to the endothelium. Lithium is able to inhibit adenylate cyclase in certain cells [6]. It can be tentatively suggested that adenylate cyclase inhibition lies at the basis of the protective action of beta-antagonists and lithium hydroxybutyrate against catecholamine-induced injury to the aortic endothelium.

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PLASMA PHOSPHOLIPASE IN BURN SHOCK

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A cardinal syndrome of burns is a disturbance of the structural and functional state of the cellular and intracellular membranes in various organs and tissues [4], associated with a change in their phospholipid composition [2].

This change may be brought about by a change in activity of lipid peroxidation (LPO) or endogenous phospholipase activity.

There are data in the literature on intensification of LPO in burns, but all that is known about phospholipases is that their high activity is observed in the blood, urine, and wound exudate of patients with severe burns during the first weeks [3].

To assess the role of phospholipases in the formation of the basic burn syndromes and, in particular, the syndrome of generalized damage to biomembranes, it is useful to study phospholipase activity in the earliest period of burns.

The aim of this investigation was to study plasma phospholipase activity in patients with burn shock and also in rats during the first hours and minutes after an experimental burn.

Phospholipase activity was compared with plasma trypsin activity and with the intensity of LPO, in view of data indicating conversion of the inactive precursor of phospholipase A into the active form of the enzyme through the action of plasma trypsin on the precursor [12] and on activation of phospholipase by factors initiating LPO [9].

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