EFFECT OF ISOPRENALINE ON AGGREGATING AND ADHESIVE PROPERTIES OF CIRCULATING NEUTROPHILS IN RATS

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Isoprenaline (IP), a synthetic β-adrenoreceptor agonist, is used to create a model of cardiomyopathy in rats [2]. Lesions produced in the wall of the heart by IP are evidently due to the cytotoxic effect, characteristic of catecholamines. According to Severin and co-workers [12], application of IP leads to death of cardiomyocytes. However, with respect to certain features the pathological manifestations in the heart in cardiomyopathies caused by catecholamines are similar to changes observed in the peri-infarct zone [3]. An important role in the pathogenesis of myocardial infarction is played by neutrophilic polymorphonuclear leukocytes (PML), which are powerful sources of toxic forms of oxygen: the superoxide-anion etc. [6]. The cytotoxic effects of these substances are well known [14]. Premedication of animals with a mixture of superoxide dismutase and catalase significantly reduces the severity of the post-infarct lesions; the same effect, moreover, can be achieved by injection of neutrophilic antiserum [9].

The role of PML in damage to heart muscle in isoprenaline-induced cardiomyopathy has not been studied. One possibility is that catecholamines can exert an activating effect on leukocytes circulating in the coronary vascular bed, and can thus involve them in the pathological process. The earliest signs of a change in the functional state of the leukocytes under the influence of a stimulating factor is aggregation of the cells and their adhesion to the endothelial lining of the vessels [8]. These indicators were used in the present investigation to assess the effect of IP on the state of the neutrophils in the case of local and systemic administration of the drug.

EXPERIMENTAL METHOD

To study the effect of IP on the aggregating properties of leukocytes, noninbred albino rats were given a subcutaneous injection of a 1% solution of IP in a dose of 80 mg/kg body weight. Control animals were given an injection of physiological saline. Under open ether anesthesia, 2, 4, 6, and 12 h after infusion, blood was taken from the heart and stabilized with heparin (20 IU/ml). PML were isolated in a double Ficoll-Verografin density gradient [15]. The cells were washed and suspended in Hanks' solution in a concentration of 10^6 cells/ml. Viability, by the trypan blue test, was 95-98%. The state of aggregation of the cells was studied by means of a turbidimetric system, based on the KFK-2 photoelectric colorimeter at 37°C, with constant mixing. The leukocytes were stimulated by addition of a solution of arachidonic acid in a final concentration of $2.5 \cdot 10^{-4}$ M. The aggregation response was assessed by measuring the transmittance of the suspension from the time of addition of the activator until the aggregatogram flattened out on a Plateau (Fig. 1).

The adhesive properties of PML were studied by intravital microscopy of the mesentery of the rat small intestine. Under pentobarbital anesthesia (50 mg/kg) laparotomy was performed, loops of small intestine with segments of the mesentery in the region of the ileocecal angle were removed, and were straightened out on a plexiglas stage. The prepara-

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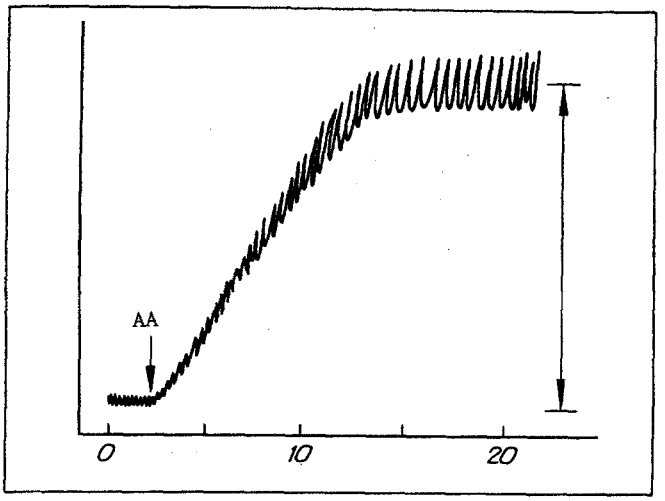


Fig. 1. Aggregatogram reflecting time course of aggregation of PML induced by arachidonic acid (AA). Final AA concentration $2.5 \cdot 10^{-4}$ M. Ordinate, transmittance (in relative units); abscissa, time (in min).

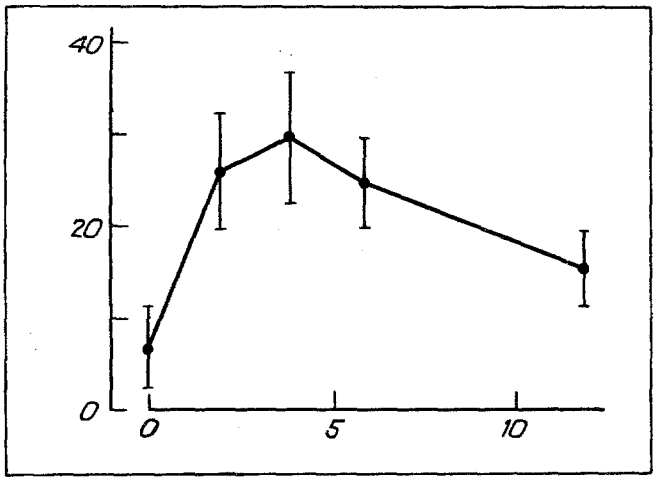


Fig. 2. Changes in arachidonate-stimulated aggregation of PML after subcutaneous injection of IP into rats in a dose of 80 mg/kg. Ordinate, transmittance (in relative units); abscissa, time (in h).

tion was constantly irrigated with Hanks' solution (pH 7.4), heated to 37°C. Exposure of the mesentery did not exceed 0.5 h, thus avoiding spontaneous microcirculatory disturbances. The microvessels were examined under a large "Leitz" microscope for intravital investigations in transmitted light (objectives ×20 and ×55). The phenomena observed were recorded on NK-2 film by means of an "Orthomat" camera. The materials were developed under standard conditions. The number of cells fixed to the walls of the vessels was estimated by the method in [4] in two groups of animals. The animals of group 1 received a preliminary injection of IP in a dose similar to that used to study aggregation. The state of the circulating leukocytes was analyzed also 2, 4, 6, and 12 h after injection. In group 2, the number of leukocytes adherent to the walls of venules was counted in a part of the vessel, chosen arbitrarily, 100 μ long before and after application of 0.1, 0.01, and 0.001% solutions of IP for 5 min to the surface of the mesentery.

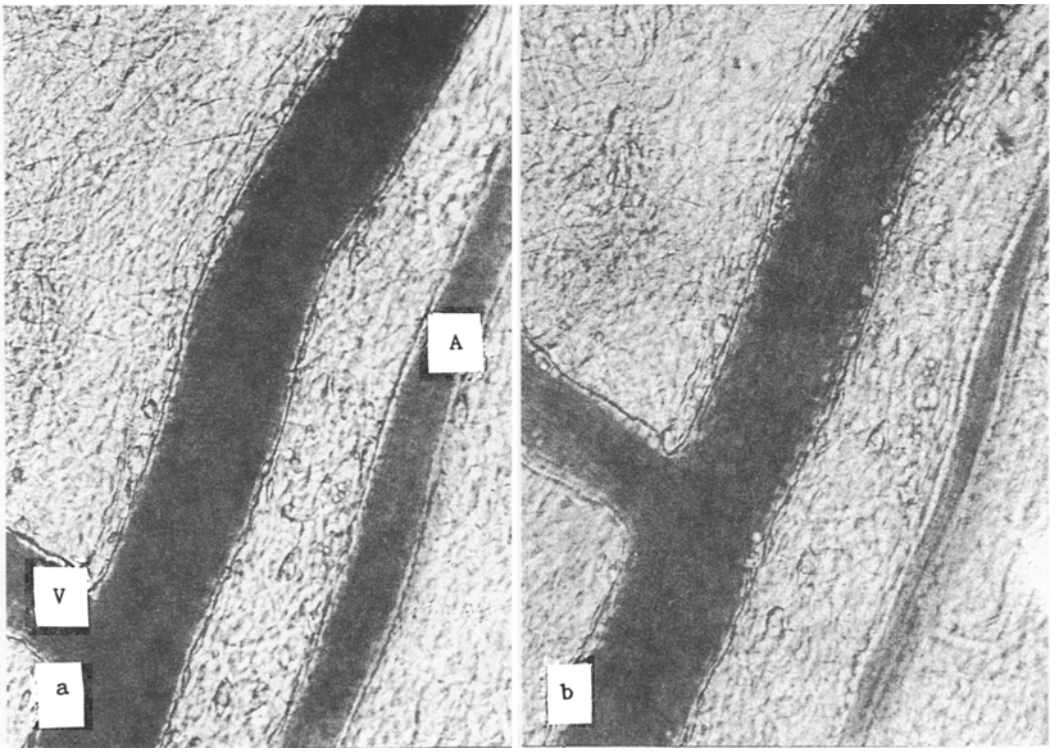


Fig. 3. Effect of application of IP solution to microcirculation in mesentery of rat small intestine: a) state of microvessels before application; b) spasm of arteriole, slowing of blood flow, and increase in number of leukocytes fixed in venules. Objective ×20.

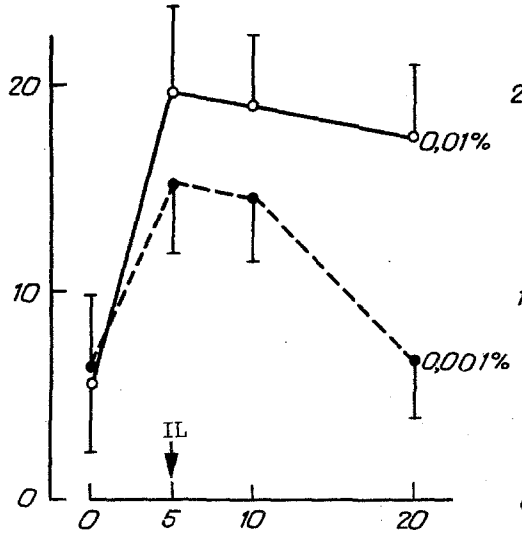
EXPERIMENTAL RESULTS

After incubation of PML isolated from the blood of intact animals, arachidonate-stimulated aggregation of the cells in solution containing the catecholamine was almost identical with the control (7.2 ± 4.5 and 6.6 ± 4.0 relative units respectively). In the case of preliminary injection of the drug into the animals the functional state of the cells showed a marked change. The aggregation index 2-4 h after infusion became 3-4 times higher than in the control (27.3 ± 6.4 relative units). With a further increase in the period of circulation the response of the cells to the stimulator gradually decreased (Fig. 2).

The intravital observations show that application of 0.1% IP solution to the surface of the mesentery causes constriction of arterioles and slowing of the blood flow. The flow of erythrocytes in the vessels becomes striated in appearance (Fig. 3). These changes disappear 5-10 min after the end of exposure, but the number of leukocytes fixed to the luminal surface of the venules remained high until the end of the period of observation. In response to application of 0.01% IP solution the vasomotor response appeared weak and became virtually invisible when a 0.001% concentration of IP in the solution was used. The character of the response of the leukocytes also depended on the catecholamine concentration. When a 0.01% concentration was used the number of fixed cells exceeded the control level until the end of the period of observation. The least concentrated solution gave the opposite reaction: the number of leukocytes on the vessel walls 15-20 min after the end of application became close to that before the beginning of application (Fig. 4).

An increase in the number of cells fixed to the walls of the venules also was observed after intravenous injection of IP. The number of adherent leukocytes 4 and 6 h after the beginning of injection was 2-3 times greater than in the control (Fig. 5). After circulation of the drug for a longer period (12 h) activity of cells was less marked, but it still differed significantly from the control (9.3 ± 2.6 and 6.5 ± 2.2 cells in a segment of the vessel 100 μ long respectively).

The results of this analysis show that IP, injected into the blood stream and also applied to the surface of the mesentery, can induce an increase in adhesiveness of the leukocytes, as shown by an increase in the number of cells fixed to the vessel walls and, in experiments in vitro, an increase in their aggregating ability. These changes follow a similar time course and they reflect different manifestation of the cell activation process [8]. However, the effects described above



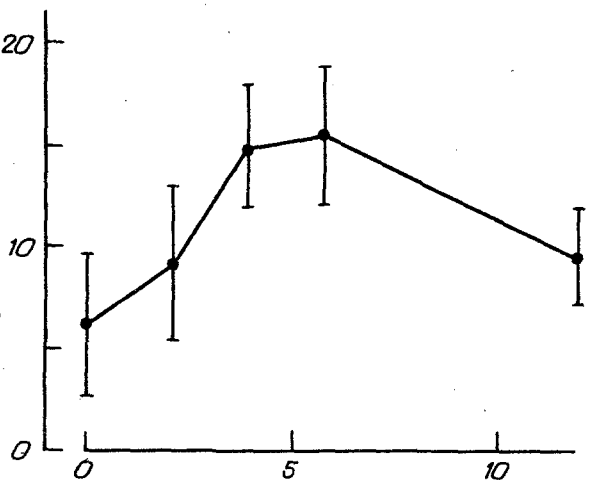


Fig. 4 Fig. 5

Fig. 4. Effect of different IP concentrations on adhesion of leukocytes to luminal surface of endothelium of venules. Ordinate, number of leukocytes on arbitrarily chosen part of vessel 100 μ long; abscissa, time (in min).

Fig. 5. Change in adhesive properties of PML after infusion of IP (80 mg/kg). Ordinate, number of leukocytes on arbitrarily chosen part of vessel 100 μ long; abscissa, time (in h).

cannot be interpreted as the result of a direct effect of the drug on PML, for arachidonate-stimulated aggregation of cells taken from intact animals was unchanged in the present of the catecholamine. It can be tentatively suggested that phenomena induced by infusion of IP are evidently linked with the action of certain secondary factors. An increase in the pool of these substances in the plasma or locally is possible as a result of changed functions of the endothelial cells. Under the influence of adrenoblockers the endothelial cells have been shown to undergo morphological transformations, which vary depending on the dose used, and which may lead to irreversible consequences [1]. These changes are nonspecific in character and at a certain stage of development they may be connected either with release of chemical attractants, which are synthesized by cells of the endothelium [10], or with oxidation of membrane lipids and the formation of arachidonic acid metabolites — leukotrienes [5].

Another pathway of activation of PML may be indirectly through the complement system [11]. Active forms of oxygen, formed during spontaneous oxidation of the catecholamine, may act as inducers of formation of fragments causing changes in the state of the neutrophils (C_{3b}, C_{5a}) [13]. Production of these agents may take place more rapidly in the presence of enzymes and metallic ions [7].

It can be concluded from the facts described above that the development of a cardiomyopathy, induced with the aid of isoprenaline, may be based not only on receptor-dependent damage to heart muscle cells, but also on destructive processes linked with the appearance of leukocyte activation products.

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POSTISCHEMIC LIPID PEROXIDATION AND MYOCARDIAL CONTRACTILITY DEPENDING ON LEVEL OF HYPOTHERMIA PROTECTING THE HEART

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Deep cooling of the myocardium is one of the most important factors for protection of the heart muscle against ischemia during open heart operations [2, 5]. However, hypothermia has a twofold action on cellular structures: on the one hand it lowers the level of metabolism and thereby prevents the development of ischemic lesions connected with a disturbance of energy metabolism [4]; on the other hand, by changing the phase state of lipids, it can induce structural and functional disturbances of the cell membranes. These changes, moreover, only become evident after reperfusion (RP) of the heart [8]. One of the systems maintaining the lipid composition of membranes and its physicochemical parameters within an assigned physiological range is lipid peroxidation (LPO). Accordingly, the aim of the investigation described below was to compare LPO and cardiac contractility during ischemia and RP and their dependence on the level of hypothermia used to protect the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on isolated hearts of noninbred albino rats weighing 180-220 g, anesthetized with pentobarbital (25 mg/kg). The heart was perfused in the retrograde direction by the Langendorff–Falen method, with standard Krebs–Henseleit solution, aerated with a gas mixture of 95% O_2 and 5% CO_2, at 37°C and pH 7.4. A small latex balloon with constant volume was introduced into the left ventricle. The pressure inside the balloon was recorded by means of a 6MDXIIS pressure transducer. The following parameters of cardiac contractility were calculated from the pressure curve and its first derivative: the developed pressure (P_d) and end-diastolic pressure (EDP). The heart was stopped after 15 min of perfusion by simultaneous compression of the aorta and cooling of the myocardium to 8-12°C in the experiments of series I and to 4-6°C in series II. RP was carried out after 90 min of ischemia. At the 15th minute of perfusion, at the end of the period of ischemia, and after 7 and 90 min of RP the hearts were placed in liquid nitrogen in order to study LPO products. Intact hearts served as the control. The level of conjugated dienes (CD) and trienes (CT) was determined spectrophotometrically at wavelengths of 233 and 275 nm respectively in a solution of lipids, extracted by Folch's method

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