FUNCTIONAL ACTIVITY OF BLOOD POLYMORPHS IN RATS WITH ISOPROTERENOL-INDUCED CARDIOMYOPATHY

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One of the main mechanisms of damage to cardiomyocytes during the development of myocardial necrosis is activation of lipid peroxidation (LPO), which can be initiated by an increase in production of active forms of oxygen (AFO) and release of bivalent ferrous ions [9]. Among the processes responsible for AFO production in myocardial ischemia an important role is played by activation of polymorphonuclear leukocytes (polymorphs), and oxidation and synthesis of catecholamines [10]. Isoproterenol (1,3,4-dihydroxyphenylisopropylaminoethanol; IP), a synthetic catecholamine, for instance, induces myocardial necrosis similar in its course and pathomorphological manifestations to myocardial infarction induced by coronary arterial occlusion, and for that reason it is widely used in pathological models of cardiomyopathies.

Until recently the only interpretation of the cytotoxic action of IP was based on its inotropic and chronotropic effect on the myocardium and on vascular tone. Meanwhile an alternative view has now appeared, based on the direct cytotoxic effect of IP on cardiomyocytes due to the production of AFO and (or) interaction of oxidation products of catecholamines with protein SH-groups [14]. However, the maximal cardiotoxic effect of oxidation of IP is manifested only when other mechanisms of LPO activation are themselves activated: the appearance of free fatty acids, activation of polymorphs [8], and release of ferrous ions [9].

The aim of this investigation was accordingly to study the dynamics of changes in parameters of polymorph function: the intensity of luminol-dependent chemiluminescence and aggregation, and also LPO of blood plasma, and homogenates of myocardium and liver, during cardiomyopathy formation following administration of IP to rats.

EXPERIMENTAL METHOD

Experiments were carried out on 58 albino rats weighing 200-250 g. IP was dissolved in physiological saline with 1% concentration and injected subcutaneously in a dose of 80 mg/kg body weight. At intervals of 2, 4, 6, 12, 24, and 48 h after injection of IP the rats were anesthetized with ether and about 10 ml of blood was taken from the heart and stabilized with heparin (20 U/ml); the heart and liver of the animals also were removed and homogenized. The quantity of TBA-active products was determined [6] in the homogenate and blood plasma. Polymorphs were isolated by the method in [7]. To record luminol-dependent chemiluminescence (LCL) 10^5 neutrophils and 10^{-6} M luminol were used. The volume of the cuvette was 1 ml. The cells were stimulated by addition of 40 μl of a suspension containing 0.8 mg/ml of $BaSO_4$ particles.

Aggregation of polymorphs was determined on a turbidimetric apparatus, built on the basis of a photocolorimeter, at 37°C with constant mixing. Polymorphs (10^6) in Hanks' solution were introduced into the cuvette in a volume of 1 ml. Aggregation of the cells was stimulated by addition of arachidonic acid solution to a final concentration of $2.5 \cdot 10^{-4}$ M. To determine the relative number of granulocytes in the leukocytic fraction of blood cells, a "Minos STX" automatic cell counter ("ABX," France) was used.

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TABLE 1. Changes in Functional Activity of Granulocytes and Quantity of MDA Products During Development of IP-Induced Cardiomyopathy in Rats ($M \pm m$)

Parameter measured	Control	Time after injection of IP, h						
		2	4	6	12	24	36	48
Proportion of granulocytes in leukocytic formula of rat blood, per cent	23,2±8,2 (8)	67,0±18,0* (6)	—	37,3±10,1* (6)	37,7±12,2* (6)	22,3±13,3 (6)	—	—
Intensity of LCL of polymorphs, conventional units, in response to stimulation by $BaSO_4$	29±14 (12)	116±26* (6)	—	98±22* (6)	81±18* (6)	60±27* (6)	—	—
Change in light transmission of polymorph suspension 15 min after stimulation by arachidonic acid, conventional units	12,2±4,0 (12)	30,4±5,4* (6)	32,0±6,0* (6)	28,6±4,8* (6)	22,0±5,1* (6)	— (6)	—	—
MDA level in heart, nmoles/mg:	41±4	—	—	—	38±6	50±5*	55±13*	79±7*
spontaneous	76±6 (6)	—	—	—	47±6 (11)	102±15* (10)	107±12* (10)	114±14* (6)
Fe^{2+}-induced								
MDA level in liver, nmoles/mg:								
spontaneous	25±4	—	—	—	26±3	23±3	20±2	23±2
Fe^{2+}-induced	39±5	—	—	—	75±12*	109±27*	30±3	29±3
MDA level in blood plasma, nmoles/ml:	(6)				(10)	(10)	(6)	(6)
Fe^{2+}-induced	2,66±0,54 (10)	—	3,64±0,61* (6)	3,69±0,56* (6)	4,46±0,7* (6)	3,64±0,58* (6)	—	3,22±0,66* (6)

Legend. Number of experiments shown between parentheses. *$p < 0.05$ compared with control.

EXPERIMENTAL RESULTS

The study of the effect of IP on properties of polymorphs in experiments in vivo showed that as early as 2-3 h after injection of IP into the rats the intensity of LCL of the granulocytes reached its maximum, after which it gradually declined until the 24th hour. One of the main causes of the increase in intensity of LCL of the granulocytes in the initial period of action of IP may be an increase in the number of circulating polymorphs and the appearance of a new, more active population of neutrophils in the blood against a background of leukocytosis [12]. The first priority was accordingly to discover whether injection of IP induces granulocytosis in the rats' blood.

Table 1 shows changes in the number of blood cells of the rats before and after injection of IP. It can be seen that 2 h after injection of IP there was an increase in the relative percentage of granulocytes in the blood leukocyte formula, which had a tendency to return to normal after 24 h. Comparison of data in Table 1 leads to the conclusion that the maximal LCL-response of the polymorphs coincides in time with the trend of the granulocytosis. At the same time, the fact must be recalled that during measurements in the cuvette of the luminometer the same number of polymorphs was always taken, and consequently, maximal functional activity of the granulocytes in vivo toward the second hour of action of IP was due also to qualitative changes in the properties of the polymorphs, which may be realized in the appearance of a new and more active polymorph population in the circulating blood of the rats against the background of granulocytosis.

It is well known that the first step in infiltration of polymorphs into an inflammatory focus is their adhesion to the vascular endothelium [8], and aggregation of granulocytes, especially in small vessels, may be the cause of significant disturbances of the microcirculation [1]. Investigation of the dynamics of polymorph aggregation revealed (Table 1) that the maximal light transmission of granulocytes isolated from the blood is observed toward the 4th hour after injection of IP into the rats, i.e., it coincided in time with the maximal LCL responses of the polymorphs. Consequently, it can be suggested that the increase in aggregation and, perhaps also, adhesion of neutrophils to the vascular endothelium toward the 2nd-4th hour after injection of IP may, on the one hand, cause damage to the endothelial cells, and substantial disturbances of the microcirculation, and on the other hand, it may facilitate infiltration of activated granulocytes into the ischemic region of the myocardium [1].

To assess the possible role of blood polymorphs in the initiation of LPO [2] and in the appearance of necrosis in the myocardium, it will be useful to compare the parameters of functional activity of granulocytes and LPO of the myocardium and blood plasma as functions of time. The LPO level was assessed on the basis of accumulation of TBA-active products. Clearly the

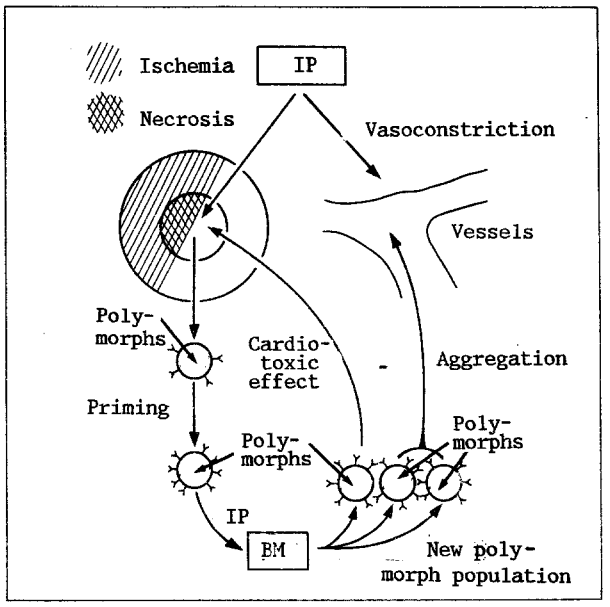

Fig. 1. Plan of activation of polymorphs during IP-induced cardiomyopathy in rats. BM) Bone marrow.

content of TBA-active products in the blood plasma increased up to a maximum 12 h after injection of IP into the rats, whereas in the case of the myocardial homogenate, the corresponding parameters of LPO had a tendency to decrease toward the 12th hour, but peak values were reached 48 h after injection of IP (Table 1). Comparison of the time of appearance of the maximal values of parameters of polymorph function and LPO of the blood plasma and cardiac homogenate indicates that polymorphs are the most mobile system in response to the action of IP.

It can accordingly be postulated that the course of events taking place in the rat after injection of IP, and leading to the onset of myocardial infarction, is as follows (Fig. 1).

1. Injection of IP, because of its inotropic vasoconstrictor effect and (or) its oxidation [13], may induce the formation of foci of microcytolysis and contractures in the myocardium [5, 11]. Release of breakdown products of cardiomyocytes and lipid metabolites into the circulation causes prestimulation of the original polymorphs [3] and the release from depots of a new and more active granulocyte population, manifested as the granulocytosis which was demonstrated.

2. The vasoconstrictor effect of oxidation of catecholamines on the blood microcirculation will be aggravated by increased adhesion and aggregation of polymorphs.

3. Chemotaxis of the activated polymorphs, their infiltration into the inflammatory focus, and maximal production of AFO and other pro-oxidants directly in the zone of necrosis initiate LPO. If it is recalled, additionally, that activity of endogenous antioxidants is sharply depressed not only in the region of the infarct, but also in the pre-infarct zone [4], the possible role of activated phagocytes in the genesis and development of the zone of necrosis becomes understandable.

LITERATURE CITED

1. G. G. Avtandilov and N. I. Yabluchanskii, Byull. Éksp. Biol. Med., **94**, No. 8, 114 (1982).
2. G. I. Klebanov, Yu. A. Vladimirov, L. U. Benov, and S. R. Ribarov, Byull. Éksp. Biol. Med., **105**, No. 6, 674 (1988).
3. G. I. Klebanov, M. V. Kreinina, V. M. Pozin, et al., Byull. Éksp. Biol. Med., **106**, No. 9, 297 (1988).
4. V. Z. Lankin, A. Kh. Kogan, A. L. Kovalevskaya, et al., Byull. Éksp. Biol. Med., **93**, No. 5, 58 (1982).
5. O. G. Yavorskii, Problems in Allergy [in Russian], L'vov (1983), pp. 118-119.
6. P. W. Albro, J. T. Corbett, and J. L. Schroeder, J. Biochem. Biophys. Meth., **13**, No, 3, 185 (1986).
7. A. Böyum, Scand. J. Clin. Lab. Invest., **21**, Suppl. 97, 77 (1968).
8. R. Engler, M. Dahlgree, M. Peterson, et al., Am. J. Physiol., **251**, No, 1, 93 (1986).
9. D. E. Gannon, J. Varani, H. Phausem, et al., J. Lab. Invest., **57**, No. 1, 37 (1987).
10. D. J. Hearse, A. S. Manning, J. M. Downey, and D. M. Yellon, Acta Physiol. Scand., Suppl. 548, 65 (1986).

11. G. Rona, C. I. Chappel, and T. Balazs, Arch. Path., **67**, 443 (1959).
12. B. Seligman, T. Chused, and J. Gallin, J. Immunol., **133**, No. 5, 2641 (1984).
13. E. Severin, S. Sartore, and S. Schiaffino, Experientia, **33**, 1489 (1977).
14. E. M. Steen, A. A. Norouna-Dutra, and N. Woolt, Pathology, **137**, No. 1, 167 (1982).

SEX DIFFERENCES IN ESTROGEN RECEPTOR ACCUMULATION IN LIVER CELL NUCLEI AND INCREASE OF BLOOD PLASMA ANGIOTENSINOGEN CONCENTRATION AFTER INJECTION OF LOW DOSES OF SYNTHETIC ESTROGENS IN RATS

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The writers previously showed the existence of sexual differentiation of the number of estrogen receptors (ER) in rat liver cytosol and described an endocrine mechanism of its formation and maintenance, based on the negative regulatory action of androgens and the direct regulatory stimulating effect of pituitary somatotrophic hormone [4]. The aim of the present investigation was to study the role of sex differences in the ER level in hepatocytes in the realization of the direct effects of estrogens in the liver of male and female rats. The experimental model for the study of this problem consisted of angiotensinogen (AG). In this decision we were guided by the following considerations. First, it can be taken to be convincingly proved that estrogens have a direct stimulating effect on its production, and that ER of hepatocytes play a key role in the realization of this effect [1, 5, 7, 9, 11]. Second, the plasma AG level in rats is not initially sexually differentiated [1, 5]; this state of affairs facilitates the recordings of differences in the degree of the stimulating action of estrogens on this parameter (if such differences are in fact observed in male and female rats). To stimulate AG production by the liver we used the synthetic estrogens hexestrol (HE) and ethinylestradiol (EE_2), in doses (0.5 and 1 μg) corresponding to physiological concentrations of the natural hormone. Synthetic estrogens, not readily metabolized, were used with the aim of eliminating the "contribution" of enzymes responsible for metabolism of sex steroids and, in particular, of estrogen-binding protein, in the development of the specific reaction.

The question of the presence of sexual differentiation of the reaction of AG production in response to estrogens is interesting because female sex steroids are used therapeutically not only in women, but also in men. It has been shown, for instance, that the use of synthetic estrogens in prostatic carcinoma in men leads to a change in the plasma AG level [6]. It has been known for quite a long time that estrogens play a role in the system maintaining the immune response, and that they have a protective role against radiation damage [8, 10]. The question of the use of estrogens to modulate immunogenetic processes in various immunodeficiency states and in radiation leukopenia is likely to prove highly topical at the present time [12].

EXPERIMENTAL METHOD

Experiments were carried out on male and female noninbred rats. The gonads and pituitary gland were removed 3 weeks before the beginning of the experiment. HE and EE_2 (Sigma, USA) for injection were dissolved in propylene-glycol and injected

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