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ROLE OF LEUKOCYTES IN HEMATOVASAL REGULATION OF THE CIRCULATION

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KEY WORDS: polymorphonuclear leukocytes; vasoconstriction; platelet aggregation; formyl-methionyl-leucyl-phenylalanine

The blood cells have been shown to play a direct role in the intimate mechanisms regulating the regional circulation of the blood and, in particular, in the pathogenesis of its disturbances [2]. On the basis of research in this field the hypothesis of hematovasal regulation of the regional circulation has been proposed, according to which changes taking place in it are the result of combined changes in the state of the vascular network and of the blood flowing along it; these changes, moreover, may be interdependent. The role of platelets and erythrocytes has been relatively well studied in this context, but that of leukocytes has received far less study. Yet is was suggested a long time ago that leukocytes may influence the circulation of the blood not only mechanically, but also through biochemical action on elements of the blood – vessel wall system [3]. This possibility appears even more probable after discovery of synthesis of a platelet activating factor [5], of vasoactive metabolites of arachidonic acid [6], and of several highly active protein regulators [9] in leukocytes. As yet, however, the concrete role of leukocytes in regulation of the functional state of the vascular network and blood cells, especially platelets, has not yet been explained.

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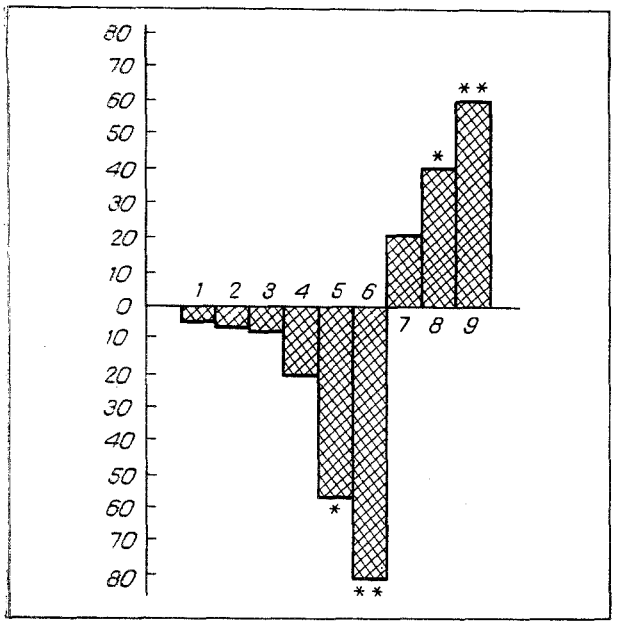

Fig. 1. Effect of infusion of $5 \cdot 10^{-8}$ M prazocin (1), $5 \cdot 10^{-6}$ M methysergide (2), 10^{-5} M aminophylline (3), 10^{-5} M cavinton† (4), 10^{-9} M prostacycline (5), $5 \cdot 10^{-6}$ M nifedipine (6), 10^{-7} M noradrenalin (7), $8 \cdot 10^{-7}$ M PGF_2 (8), and 10^{-9} M vasopressin (9) on the vasoconstrictor effect of PMNL. Ordinate, changes in effect of PMNL (in % of initial value). $^*p < 0.05$, $^{**}p < 0.01$.

The aim of this investigation was to study influences of human polymorphonuclear leukocytes (PMNL) on blood vessels and platelets.

EXPERIMENTAL METHOD

Experiments were carried out on blood from 40 healthy donors, stabilized with citrate. The PMNL fraction was obtained by the method in [4] and isolated leukocytes were resuspended in buffer to a concentration of $5 \cdot 10^6$ cells/ml. The PMNL were activated with formyl-methionyl-leucyl-phenylalanine (FMLP, 10^{-7} M), which induces marked secretion of leukocytic granules with activation of cell metabolism [7]. Preliminary investigations showed that the PMNL concentration used under our conditions has no significant effect on vessels or platelets. The vascular effects of activated PMNL were studied on models of the perfused cat carotid artery and human middle cerebral artery. Perfusion was carried out by "Watson-Marlon" peristaltic pumps with parallel recording of the perfusion pressure by "Elema" electromanometer and of the diameter of the vessel by means of a model 275 K diameter measuring apparatus (HSE, West Germany) [10]. The vascular preparation was denuded of its endothelium by the method in [8], and the degree of denudation was verified morphologically. The PMNL suspension or its filtrate was injected under bolus conditions before and after activation into the channel of the perfusion system. The effect of $PGF_{2\alpha}$ ($5 \cdot 10^{-6}$ M) was taken as the unit when the degree of their vasoconstrictor effect was studied. To investigate the effect of physiologically active substances and drugs on the vasotropic effects of PMNL, a vascular segment was perfused for 5-10 min with a solution containing the test agent, after which the PMNL suspension was injected against this background. The concentrations of these substances are given in the caption to Fig. 1. Platelet aggregation in platelet-enriched plasma (PEP) and whole blood was investigated on a "Chrono-Log" aggregometer (England). Because of the electrodynamic method on which the working of this instrument is based, the effect of changes in turbidity of the medium and other types of interference created by addition of the PMNL suspension or its filtrate to the sample could be ruled out. The results were subjected to statistical analysis by the nonparametric Wilcoxon–Mann–Whitney test and by Wilcoxon's paired test.

†Ethyl ester of apovincamic acid – Translator.

EXPERIMENTAL RESULTS

Analysis of the vascular effects of PMNL showed that their activation by FMLP is accompanied by the release of compounds whose combined effect is manifested as a vasoconstrictor reaction of the perfused vessel. It was observed after addition both of the suspension of PMNL and of their filtrate to the perfusion solution, but in the latter case the effect was 30-40% weaker. This was evidently due to additional mutual activation of the PMNL during movement in the perfusion system and their interaction with the vascular preparations. A particularly interesting fact in this connection is that after denudation of the vascular endothelium, the vasoconstrictor effect of the PMNL suspension was considerably increased. Whereas on the intact vessel the PMNL suspension induced a vasoconstrictor effect measuring 1.30 ± 0.25 relative units, after denudation of the endothelium the effect measured 2.25 ± 0.37 relative units ($p < 0.01$). This may be due to abolition of the production of vasodilator agents, namely prostacycline and endothelial vasodilating factor, limiting the constrictor effect of PMNL in the latter case.

The study of the mechanisms of the vascular effects of PMNL showed that neither prazocin, an α_1-adrenoblocker, nor methysergide, a serotonin receptor antagonist, caused any significant change (Fig. 1), i.e., these types of receptors are not involved in the mechanism of their vascular effects. Meanwhile nifedipine, a calcium antagonist, depressed the vasoconstrictor effect of PMNL considerably. Prostacycline, an adenylate cyclase activator, and, to a lesser degree, the phosphodiesterase inhibitors aminophylline and cavinton, had a similar action. The mutual potentiation of the effects of prostacycline and nifedipine on the vasoconstrictor action of PMNL will be noted. Whereas in low concentrations prostacycline (10^{-10} M) and nifedipine ($5 \cdot 10^{-8}$ M) acting independently, depressed it by 15.7 ± 4.3 and 23.6 ± 4.1%, respectively, together they depressed it by 79.6 ± 8.0%. These data suggest that the vasotropic effects of PMNL are associated with the action of agents released from them on the vascular smooth muscle, with an increase in the inflow of calcium into the myoplasm of the cells. The mutual potentiation of the effects of prostacycline and nifedipine under these circumstances may be connected with the fact that the former stimulates calcium release from the myoplasm both indirectly through cAMP and directly, whereas the latter prevents the inflow of calcium into the cells.

The influence of physiological vasoconstrictors on the effect of PMNL is also interesting. It will be clear from Fig. 1 that noradrenalin, vasopressin, and PGF_2, infusion of which in the low concentrations used did not itself give rise to any significant changes in diameter of the vessels, potentiated the vasoconstrictor effect of PMNL. This positive interaction between them may be of definite importance for the mechanisms of development of angiospasm.

With their significant action on vascular tone, activated PMNL also strongly influence the state of the platelets. FMLP did not induce platelet aggregation in PEP, but did so in whole blood. Consequently, its effect in the latter case was due to activation of leukocytes. This was confirmed by the fact that addition of a suspension of PMNL or their filtrate to PEP significantly enhanced platelet aggregation. The filtrate under these circumstances enhanced aggregation of autologous platelets by 64.2%, compared with 213.5% by the suspension ($p < 0.01$). Consequently, in this case also the PMNL suspension was more active than the filtrate. This result can also be explained by the mechanical influence of leukocytes on platelet aggregation, with the formation of combined platelet-leukocytic aggregates and with mutual activation of platelets and leukocytes in the suspension by physiologically active substances released from them. It is also important to note that in the presence of PMNL not only was the aggregating ability of the platelets increased, but their sensitivity to physiological antiaggregants was modified. Thus, whereas prostacycline (2 ng/ml) inhibited platelet aggregation in PEP by 64.7%, in the presence of PMNL it did so by 15.2%.

Thus, PMNL can release agents capable of inducing considerable changes of vascular tone and of platelet aggregation into the blood stream. Hence it follows that activation of PMNL by immunologic and nonimmunologic stimuli is one factor in the development of angiospasm, thrombogenesis, and the microembolic syndrome coupled with the onset of vaso-thrombocytic dysfunctions. Because of this state of affairs new approaches to pharmacologic correction of these phenomena, associated with influences on the modulating function of leukocytes, must be developed.

It will be noted that the effects of PMNL described above are evidently the result of the action of a whole series of physiologically active substances. In certain cases, espe-

cially against the background of calcium antagonists and prostacycline, PMNL induce, not vasoconstriction, but relaxation of the vessel and inhibition of platelet aggregation. This may perhaps be the result of the action of antiaggregating and vasoconstrictor factors formed in PMNL together with substances with the opposite kind of effects on blood vessels and platelets. The possibility cannot be ruled out that under different conditions of stimulation, the action of these substances may be stronger.

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DEPENDENCE OF THE DEGREE OF STRESS-INDUCED HEART DAMAGE IN ENDOGENOUS β-ENDORPHIN LEVELS DURING PRELIMINARY ADAPTATION

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KEY WORDS: endogenous β-endorphin; adaptation; stress

Emotional-painful stress (EPS) can cause myocardial damage and can disturb the contractile function of the heart [1, 4]. Much attention has recently been paid to the study of the mechanisms of function of stress-limiting systems [5] and, in particular, of opioid neuropeptides [2].

The aim of this investigation was to study the time course of β-endorphin levels in the blood plasma and brain structures during adaptation of an animal to extremal factors and to compare the data with the degree of resistance of the heart of the adapted animals to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-180 g. A state of adaptation was produced by a series of short-term immobilizations [6] or a course of Rhodiola extracts (a preparation with marked adaptogenic properties [7]), obtained from the stonecrop Rhodiola rosea, periodically in a dose of 1 mg/kg daily for 8 days. The action of this substance in the intact animal is realized through its effect on brain energy metabolism and its modulating action on the state of the pituitary-adrenal system. The rats were decapitated after 1, 3, and 5 sessions of adaptation by each of the above methods, and also at the end of the course of Rhodiola extract (8th day) or series of immobilizations (10th day),

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