STATE OF THE MAST CELLS AND BLOOD CLOTTING DURING LONG-TERM NORADRENALIN ADMINISTRATION

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UDC 611-018.1+612.115:615.357

KEY WORDS: noradrenalin; connective tissue; mast cells; blood clotting.

Stress states accompanied by activation of the sympathicoadrenal system and by catecholaminemia play a pathogenic role in the development of pathological changes in the body in general and the heart in particular [6]. It is generally accepted that an excess of exogenous catecholamines causes necrotic changes in the myocardium. A role of great importance in the pathogenesis of these lesions is played by microcirculatory disturbances caused by a change in the state of the blood clotting system and the state of platelet function [11, 13]. However, many steps in the action of catecholamines in the body remain inadequately explained. We could find no reference to the study of the role of morphological and functional changes in connective tissue under these circumstances, although it is recognized that connective tissue is a unique stage on which all pathological processes are manifested [8]. Our choice of connective tissue mast cells as test object was determined by the fact that this cell system is a carrier and producer of heparin [14], which can exert an effective influence on coagulation and microcirculatory hemostasis.

The aim of this investigation was to study whether any connection exists between the morphological and physiological state of the mast cells and of the blood clotting system during long-term administration of a catecholamine

EXPERIMENTAL METHOD

Experiments were carried out on two groups of mongrel dogs weighing 9-18 kg: group 1 consisted of 10 intact animals (control), and group 2 of eight animals into which noradrenalin (NA) was injected in a dose of 0.56 μk/kg/min for 2 h through a catheter implanted beforehand into the jugular vein. The duration of the course was 6 days [9]. Blood was taken 24 h after the last infusion and the animals were sacrificed. Full-thickness pieces of skin were excised for morphologic investigation and fixed in 12% neutral formalin and Lillie's fluid. Films were stained with toluidine blue at various pH values. The number of mast cells was counted in the intervascular areas in 15 fields of vision (magnification 400) and the dimensions of the round and oval cells were determined. Functional activity of the mast cells was judged on the basis of their degree of maturity (under 13 μ – young forms, 13-15 μ – average, over 15 μ – mature) and the degree of degranulation (with solitary granules outside the cell – degree I, with massive escape of granules – II, completely destroyed cells – III). Blood for investigation was sampled through a catheter inserted into the jugular vein. The state of the plasma clotting factors was assessed by traditional biochemical methods. The adhesive properties of the platelets was determined by Multen's method and their aggregation properties by the method of Baluda et al. [1].

RESULTS

The morphologic observations showed predominance of oval and round mast cells in the intervascular regions of the film preparations from the control group, uniformly scattered among the other cells without any sign of grouping (Fig. 1). The mast cells stained well with toluidine blue and their granules were the same size. Evidence of degranulation was slight, and the number of mast cells varied from 40 to 90, on average 65.0 ± 4.8. Their diameter varied from 9 to 21 μ and cells from 14 to 17 μ in diameter constituted the highest percentage. The mean diameter was 15.8 ± 0.4 μ.

Laboratory of Physiology of Blood and Laboratory of Pathophysiology of Adaptation, Institute of Physiology and Experimental Pathology of High Altitudes, Academy of Sciences of the Kirghiz SSR, Frunze. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 663-666, December, 1989. Original article submitted November 17, 1988.

0007-4888/89/0012-1705$12.50

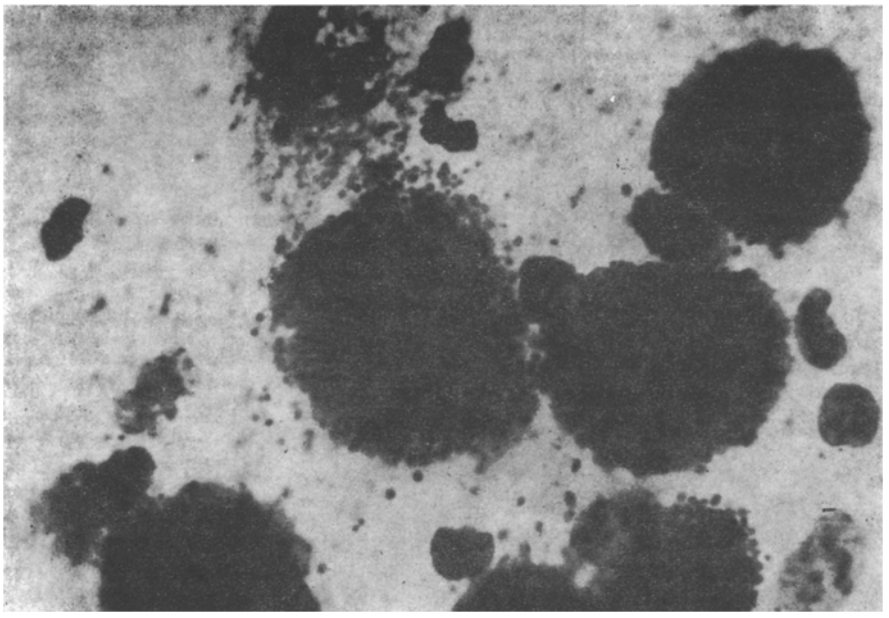

Fig. 1. Mast cells without any clear signs of functional activity (control). Here and in Fig. 2: stained with toluidine blue; 400×.

TABLE 1. Basic Parameters of Morphologic and Functional State of Mast Cells and of Blood Clotting during Long-Term Administration of NA

Parameter	Control (10)	Experiment (8)
Mast cells		
Number	65±4,8	41±3,2**
Diameter, μ	15,8±0,4	13,9±0,5*
Degree of maturity, %:		
young	18±2,1	42±5,1**
average	51±3,6	47±3,9
mature	31±4,1	11±2,6**
Degree of degranulation, %		
I	63±4,5	28±2,8**
II	26±2,8	37±1,2*
III	11±1,1	35±2,1**
Blood plasma		
Free heparin, sec	12±1,1	18±2,3*
Activated recalcification time, sec	44±3,6	32±1,9*
Maximal clotting activity, %	66±5,1	44±7,2*
Thromboplastin inactivation index, conv. units	1,49±0,08	1,2±0,05*
Factor XIII, sec	22±1,5	23±3
Fibrinogen, mg %	396±72	446±96
β-fibrinogen, mg %	60±5,8	86±6,3*
Fibrinolytic activity, %	35±6,2	48±11,7
Fibrinogen degradation products, mg %	70±9,7	159±25,9**
Maximal percentage of aggregation	78±2,8	76±6,4
Platelets		
Aggregation time, sec	288±54	340±64
Disaggregation time, sec	506±72	350±59
Adhesiveness index, conv. units	1,2±0,1	1,2±0,2

Legend. Number of experiments given in parentheses. *$p < 0.05$, **$p < 0.01$.

A common feature, constantly found, of the mast cells was the presence of metachromatic granules in their cytoplasm. The number and size of the granules depended on the degree of maturity of the cells. Large forms of mast cells (over 18 μ) with a large number of brightly stained metachromatic granules were constantly found at a distance from the blood vessels. Small cells (under 15 μ) were found in the immediate vicinity of the blood vessels.

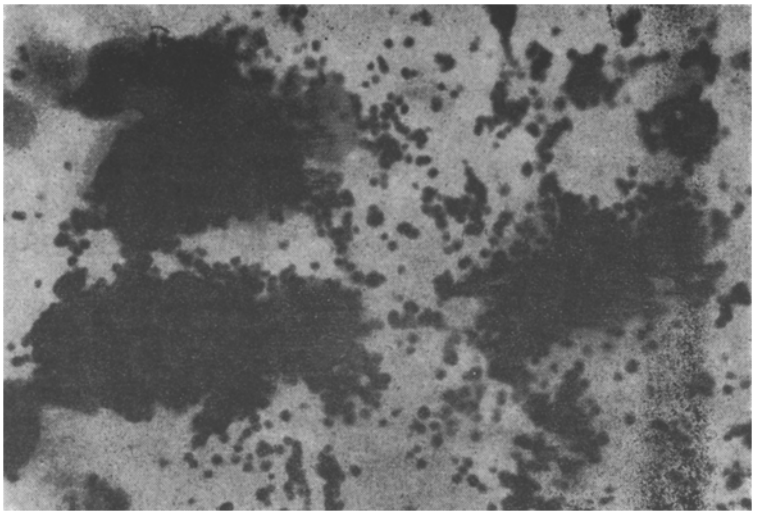

Fig. 2. Mast cells with greatly intensified functional activity and evidence of disintegration (experiment).

Administration of NA led to significant changes in the state of the mast cells. Their number fell sharply (by 37%) to 41.0 ± 3.2 compared with 65.0 ± 4.8 ($p < 0.05$) in the control group (Table 1). The decrease in size of the mast cells took place on account of the disappearance of large forms and predominance of small cells with an average diameter of 13.9 μ. The frequency of marked degranulation increased considerably, up to complete destruction of the mast cells (Fig. 2). Clumpy forms of mast cells and massive areas densely studied with metachromatic granules were frequently found. Cells which remained appeared loose in texture, they lost their inherent clarity, and the intensity of their staining was reduced. It can be tentatively suggested that the increase in the number of young forms of mast cells observed in this case was connected with destruction and death of the mature cells, i.e., of the largest forms. A tendency was observed in the experimental group of animals for mast cells to form groups and long chains (of 5-10 cells) and for the distance between them to be reduced. "Agranular" cells, resembling ghosts of mast cells, with many granules lying freely around them in the interstitial tissue, were often found. The number of vacuolated cells was increased and cells were seen with a finely granular appearance, staining more palely than usually, and giving the impression of "melting" of the granules. These changes also were a distinguishing feature of the experimental group.

Investigation of coagulation and platelet-induced hemostasis in the group receiving NA (Table 1) revealed not only hypocoagulation changes (an increase in the free heparin time, a decrease in maximal clotting activity, a considerable increase in the concentration of fibrinogen degradation products), but also evidence of intensification of the coagulation component of hemostasis (shortening of the activated recalcification time, lowering of the thromboplastin inactivation index, and elevation of the β-fibrinogen level). The aggregation and adhesive properties of the platelets were unchanged.

The experiments thus showed that during long-term administration of NA, activity of the connective tissue mast cells is enhanced and the heparin concentration rises, evidently due to release of heparin from the mast cells as a result of their degranulation and disintegration. The hyperheparinemia in this case must be regarded as a unique compensatory-defensive reaction, aimed at stimulating tissue respiration [5] and at preserving coagulation and microcirculatory hemostasis [7]. According to the data of [11], prolonged administration of NA induced an increase in platelet aggregation in dogs. Analogous results were obtained [12] in experiments on rats; artificial thrombocytopenia or the administration of prostacyclin improved the state of the microcirculatory hemostasis. In stress and during administration of the catecholamine, nonenzymic fibrinolysis was considerably intensified due to the formation of complexes with heparin, and its contribution to the total fibrinolytic activity of the blood increased to 60% [4]. Enhancement of the state of mast cell function and elevation of the free heparin level in the present experiments could facilitate the intensification of nonenzymic fibrolysis. Incidentally, it is not always possible to discover a direct connection between the state of the mast cell apparatus and the heparin concentration under the influence of hormones [2]. This is evidently due to complex hormonal interactions in the body. The fact that the blood heparin level and the state of the hemostasis systems

to some degree reflect the morphological and physiological state of the mast cells confirms our views that the mast cells are heparin producers, reorganizing cellular metabolism and exerting an antistressor and antihypoxic action, and actively influencing coagulation hemostasis [3, 8].

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EFFECT OF TEMPERATURE AND Ca^{++} ON INTERACTION BETWEEN HIGH DENSITY LIPROPROTEINS AND EPITHELIAL CELLS OF THE HUMAN SMALL INTESTINE

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UDC 612.33.014.1:577.112.856].06:612.59].085.2

KEY WORDS: high-density lipoproteins; human enterocytes.

High-density lipoproteins (HDL) are responsible for reverse transport of cholesterol from the majority of organs and tissues and supplying it to organs synthesizing steroid hormones [4]. These two functions may perhaps be mediated through a specific receptor for HDL_3. Much evidence in support of the existence of specific binding sites (receptors) for HDL on the surface of many types of cells has been obtained in recent years [1, 5, 12].

The writers showed previously that epithelial cells of the human small intestine (enterocytes) have specific binding sites on their surface for HDL_3, possessing certain properties of a classical biological receptor. Binding of HDL_2 with human enterocytes is characterized by specificity, saturation, high affinity, and reversibility, it is controlled in response to saturation of the cells with cholesterol, and it is accompanied by biological effects: internalization, intensive degradation, and dissociation of particles with the release of cholesterol from the cells and stimulation of cholesterol synthesis [13, 14].

In the investigation described below, the effect of temperature and Ca^{++} on interaction of HDL_3 with enterocytes was studied in order to obtain a fuller idea of the characteristics of the receptors mediating this interaction and to elucidate their differences from receptors for low-density lipoproteins.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimenteal'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 666-668, December, 1989. Original article submitted March 20, 1989.

0007-4888/89/0012-0000$12.50