ANALYSIS OF THE MAST CELL POPULATION DURING STIMULATION AND BLOCKADE OF THE ANTICLOTTING SYSTEM

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The effector act of the anticlotting system, responsible for reflex regulation of the liquid state of the blood, consists of liberation of plasminogen activator and heparin from the tissue depots into the circulating blood [1, 12]. Heparin, which forms complex compounds with the thrombogenic blood proteins and biogenic amines, induces nonenzymic fibrinolysis, characterized by ability to dissolve unstabilized fibrin through its dissociation into molecules of fibrin monomer [13]. In some types of pathology and also under experimental conditions, for example, under the influence of chlorpromazine, dysfunction of the anticlotting system arises [1]. On this basis prethrombosis or thrombotic complications develop, associated with thrombin formation *in vivo* [8]. The site of active synthesis of heparin, and its storage and secretion, is known to be the mast cells (labrocytes) which, besides heparin, also secrete histamine, proteases, prostaglandins, and other biologically active substances and participate both in the regulation of tissue homeostasis, affecting permeability, blood supply, and the growth and activity of cells within a narrow radius [6, 15], and also the regulation of hemostasis [10, 14]. This last function is realized by virtue of the predominantly perivascular and pericapillary distribution of the mast cells, which ensures their close contact with nerve endings in the vessel walls. The problem of the role of mast cells in the effector component of the reflex-humoral reaction of the anticlotting system has received little study [1].

To create an experimental model of stimulation of the anticlotting system usually only exogenous or endogenous thrombin (active serine protease) has been used. The discovery of structural analogs of thrombin, which preserve their specific ability to stimulate the anticlotting system but do not possess proteolytic activity, provides extensive opportunities for analysis of interaction at the ligand — receptor — nervous stimulus level, leading to an effector response through stimulation of the tissue structures storing humoral agents of the anticlotting system and, in particular, heparin.

In the investigation described below the anticlotting system was stimulated by the inactive thrombin precursor prothrombin 1 and by DIP α-thrombin, deprived of its proteolytic activity through blockade of the active center of α-thrombin by DIP† [4, 5]. The anticlotting system was blocked by chlorpromazine. The state of the anticlotting system was assessed by biochemical analysis and the state of the mast cell population by a complex morphometric approach [7].

EXPERIMENTAL METHOD

For the experiments of series I 57 male albino rats weighing 180-200 g were used. The test substances were injected into the jugular vein. Blood samples were taken (1 ml) from the same vein 5 min after injection and total fibrinolytic activity and nonenzymic fibrinoly-

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TABLE 1. Changes in Total Fibrinolytic Activity and Nonenzymic Fibrinolysis (in mm^2) after Intravenous Injection of Preparations of α-Thrombin, DIP-α-Thrombin, and Prothrombin 1 into Rats (M ± m)

Preparation injected	Total fibrinolytic activity	Nonenzymic fibrinolysis
Control (0,85% NaCl)	56±8,6 (20)	18±3,9 (20)
α-thrombin	124±10,6 (7)*	87±14,5 (7)*
DIP α-thrombin	102±6,0 (20)*	63±5,0 (20)*
Prothrombin 1	100±6,2 (10)*	63±9,2 (10)*

Legend. Number of experiments given in parentheses.
*P < 0.001.

sis determined [2]. Control animals were given an injection of 0.85% NaCl solution. The following preparations were used: α-thrombin (0.5 μM), 6 NIH units/ml, DIP-α-thrombin (0.5 μM) with clotting after-activity of under 0.1 NIH unit/ml, obtained as described in [4], and also prothrombin 1 (1.0 μM) without thrombin activity [9]. In the 10 animals of series II the reflex arc of the anticlotting system was blocked by injection of 0.06 ml of 2.5% chlorpromazine solution 15 min before injection of the α-thrombin or 0.85% NaCl solution. For morphometric analysis of the mast cell population 17 animals in the experiments of series I and eight animals in those of series II were decapitated 6 min after injection of the substances. Mast cells were studied in serous membrane preparations from the mesentery of the small intestine, omentum, renal capsule, and pericardium, fixed in buffered formalin solution and stained with 0.1% toluidine blue at pH 4.9. The PAS reaction also was carried out, but was negative in all experiments, and staining for RNA with methyl green–pyronine, which was positive in all experiments. Morphometric criteria were obtained by counting more than 800 mast cells in each animal. The combined morphometric approach [7] included the following steps: cell counts for four categories of cells, based on the number of granules and degree of metachromasia, depending on the heparin content; calculation of the heparin saturation index of the cells — the ratio of the total number of dark cells to the total number of pale cells; calculation of the degranulation index, with analysis of the relative frequency of its weak, average, and strong forms — the ratio of the total number of degranulated cells to the total number of cells. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The series of biochemical investigations showed that intravenous injection of DIP-α-thrombin and prothrombin 1, deprived of clotting activity, stimulated the reaction of the anticlotting system, as reflected by a statistically significant increase in total fibrinolytic activity and nonenzymic fibrinolysis (Table 1). The latter reflects activity of complexes of heparin with blood proteins and amines in the blood stream. The equal intensity of the effector response observed after injection of the active protease α-thrombin and of closely similar molar concentrations of its inactive forms is evidence that stimulation of the anticlotting system is determined not by the presence of proteolytic activity, but simply by the concentration of sites with high affinity for chemoreceptors of the blood stream. These sites are located outside the active center of the enzyme, in the region called the "macromolecular substrate binding (recognition) site" and which interacts with many cell receptors [11]. Hence it follows that the effector reaction of the anticlotting system, manifested as an increase in enzymic and nonenzymic fibrinolysis in the blood, is primary and is not preceded by hypercoagulation (as in the case of α-thrombin), for when these inactive forms are injected no increase is found in the soluble fibrin level in the blood to correspond to the blood thrombin level [3].

To identify the tissue sources of heparin, a morphometric analysis was made of the mast cell population of rats with different states of their anticlotting system. A series of control experiments showed (Figs. 1 and 2a) that in intact rats dark cells saturated with intensely metachromatic granules predominated; these are known to consist of a complex of

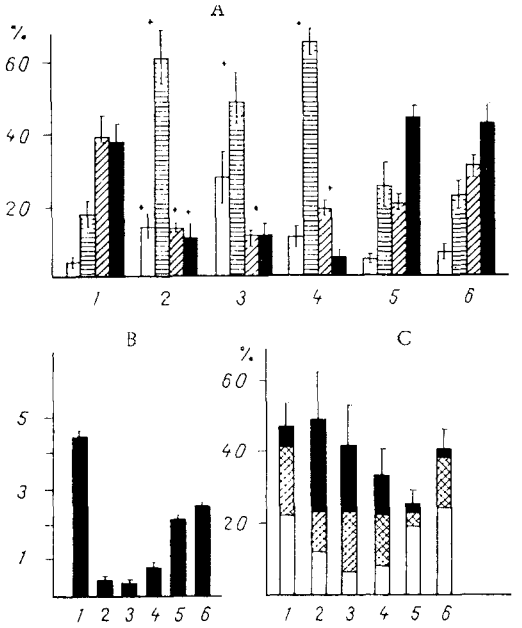

Fig. 1. Morphometric criteria of state of mast cell population of rats during stimulation (2, 3, 4) and blockade (5, 6) of anticlotting system. 1) Intravenous injection of 0.85% NaCl solution; 2) injection of α-thrombin; 3) injection of DIP-α-thrombin; 4) injection of prothrombin 1; 5) injection of chlorpromazine and 0.85% NaCl solution; 6) injection of chlorpromazine followed, 15 min later, by α-thrombin. A) Differential count of mast cell population: columns from left to right indicate relative frequency (in %) of very pale, pale, dark, and very dark cells, respectively; B) heparin saturation index; C) degranulation index and relative frequency of its forms. Unshaded part of columns corresponds to weak, cross-hatched portion to average, black portion to strong form.

*P < 0.001.

heparin with basic proteins and histamine. It seems that only mature heparin was present in the granules, for the PAS reaction was negative. The heparin saturation index of the population was 4.48 ± 1.23. Continuous secretion of the mast cells was reflected in their degranulation and granulolysis. The latter effect was reflected in the appearance of pale cells with weak metachromasia and of very pale, empty cells. Degranulated cells accounted for 46.6 ± 6.8% of the total number of cells, predominantly with a weak type of secretion, characterized by only a few granules located outside the cell membrane (Fig. 2a). In the series of experiments with intravenous injection of substances specifically stimulating vascular chemoreceptors, not possessing clotting activity, but inducing a response of the anticlotting system, a substantial shift of the mast cell population toward pale forms was observed. The action of DIP-α-thrombin and prothrombin 1, brought about through the appearance of a nervous stimulus, was similar and was characterized by a significant ($P < 0.01$) decrease in the intracellular heparin level, reflected in an increase in the number of pale and empty cells (Figs. 1 and 2c, d), and by a sharp fall in the heparin saturation index below unity. These changes correlated completely with the picture of changes in the mast cell population after stimulation of the anticlotting system with α-thrombin (Figs. 1 and 2b). No significant change was found in the degranulation index in these experiments (Fig. 1C), but its character was changed on account of a marked increase in the strong type. Apocrine secretion of granules by exocytosis evidently takes place. Secretion of heparin by mast cells in response to stim-

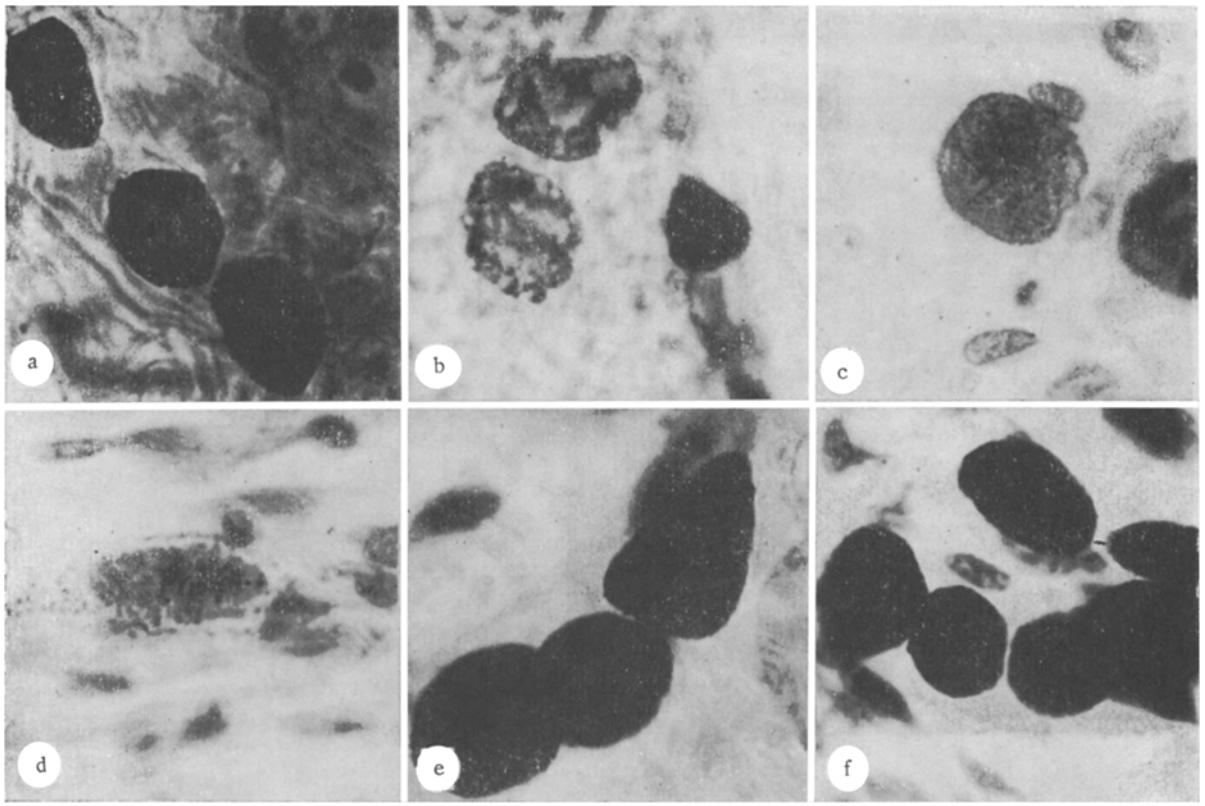

Fig. 2. Mast cell population during stimulation (b, c, d) and blockade (e, f) of anticlotting system: a) injection of 0.85% NaCl solution; b) injection of α-thrombin; c) injection of DIP-α-thrombin; d) injection of prothrombin 1; e) injection of chlorpromazine and 0.85% NaCl solution; f) injection of chlorpromazine and α-thrombin. Toluidine blue, 400 ×.

ulation of the anticlotting system, however, takes place mainly on account of granulolysis, the level of which was significantly (3-4 times) higher in the experimental groups than in the control. This corresponds to merocrine secretion, in which lysis of the granules and liberation of the products through the intact membrane take place. Pale vacuoles appear in the cell and a metachromatic halo around it.

In the next series of experiments α-thrombin was injected into animals in which the reflex arc of the anticlotting system was blocked by chlorpromazine. No changes characteristic of the response of the anticlotting system were observed in the animals' blood, confirming previous observations [3]. Blockade of the anticlotting system and injection of α-thrombin against this background caused no significant changes in the mast cell population ($P > 0.5$, Fig. 1). As a result of a small decrease in the number of dark and an increase in the number of pale cells the heparin saturation index fell, but emptying of the mast cells did not arise (Fig. 2e, f). The degree of granulolysis differed only a little from the control. Degranulation took place, just as in the control, mainly on account of weak forms (Figs. 1 and 2).

This investigation showed that increased secretion of heparin by mast cells is incorporated into the effector act of the anticlotting system in response to stimulation of its proteolytically inactive forms, namely DIP-α-thrombin and prothrombin 1. This suggests that, besides a mechanism of local secretion, responsible for maintaining tissue homeostasis, reflex regulation of the function of the mast-cell population also exists and is responsible for maintaining the liquid state of the blood.

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MICROSPECTROPHOTOMETRY OF NUCLEAR DNA IN LYMPHOID CELLS OF LYMPH NODES IN SYSTEMIC BLOOD DISEASES

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Because of difficulty in the diagnosis of histological preparations of lymph nodes from patients with systemic blood diseases when ordinary methods of investigation are used, an attempt was made by microspectrophotometry to study the nuclear DNA content of lymphoid cells from patients with chronic lymphatic leukemia, myelomatosis, and a malignant lymphoma of non-Hodgkin type, and the results are given below [2-4].

EXPERIMENTAL METHOD

Microspectrophotometry was carried out on a scanning integrating digital microspectrophotometer, an improved version of the instrument which scanned an enlarged image of the object in two mutually perpendicular directions [1], and which differs from the prototype by scanning the image along lines 0.5 μ wide in frames measuring 5 × 5, 10 × 10, 15 × 15, and 20 × 20 μ^2. Scanning of the image along lines was linear; its sweep could be changed in discrete steps corresponding to the frame chosen by the investigator from the control panel. The frame was scanned in strips in stages with a 0.5-μ step. The scanning time was 5-20 sec. The signal from the photomultiplier for each line was led to a matching preamplifier, then to a logarithmic converter, converting optical transmission into optical density. From the output of the logarithmic converter the signal was led to an integrator, integrating optical density for each line, and at the end of scanning, giving out the total signal, proportional to the integral optical density of the biological object analyzed in the frame, and corresponding to the quantity of test material in it. The analog signal from the output of the integrator was transformed by means of an analog-to-digital converter into a digital signal. Unlike other known scanning devices, the instrument provides for automatic compensation of

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