HEPARIN SECRETION BY MAST CELLS AS AN INDICATOR OF THE STATE OF THE ANTICLOTTING SYSTEM

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Mast cells (labrocytes) participate in the regulation of hemostasis [9] by secreting, as the main component, heparin [8], and also histamine and proteins, from their granules in response to various stimuli. The writers showed previously [3] that increased heparin secretion by mast cells is effected by the anticlotting system in response to its excitation both by α-thrombin (EC 3.4.21.5) and by its analogs: DIP-α-thrombin and prethrombin 1, which do not exhibit proteolytic activity. It has also been shown [10] that the nonclotting fibrinogen β/γ-thrombin, which has weak proteolytic activity against some secondary substrates of α-thrombin, does not excite the function of the anticlotting system. The powerful proteinase trypsin (EC 3.4.21.4), homologous to α- and β/γ-thrombin in the structure of its active center, like β/γ-thrombin cannot specifically activate the function of the anticlotting system [6]. This is evidently due to the absence of a recognition site for high-molecular-weight substrates, responsible for interaction with receptors of platelets and endotheliocytes, in the structure of trypsin and its disturbance in β/γ-thrombin [4, 7]. The question arises, whether proteinases of trypsin type, appearing in the blood and not interacting with chemoreceptors of the vessel wall, have any influence on the state of the mast cell population or, in other words, whether considerable intensification of heparin secretion by mast cells may be an indicator of the state of the anticlotting system.

The aim of this investigation was to compare the state of the mast cell population of rats after intravenous injection of trypsin or α-thrombin into the animals in closely similar molar concentrations. The dynamics of heparin secretion by mast cells during activation of the anticlotting system also was studied.

EXPERIMENTAL METHOD

Altogether 129 male albino rats weighing 180-200 g were used. The test substances were injected into the jugular vein. Blood (1 ml) was taken from the same vein 1, 5, 30, and 120 min after injection of the substances and total fibrinolytic activity, nonenzymic fibrinolysis [1], and the thrombin time were determined. Not more than 2 ml of blood was taken from each animal. Control animals were given injections of 0.85% NaCl solution. The α-thrombin used in the experiments had a clotting activity of 1500-2000 NIH units/mg protein and an esterase activity of 12-14 μmoles BAMÉ/min/mg protein, obtained as described in [5], and trypsin (Spofa), with esterase activity of 20 μmoles BAMÉ/min/mg protein were used in the experiments. The animals were given injections of α-thrombin or trypsin in a concentration of 10 μM (1 ml). For morphometric analysis of the mast cell population, the animals were decapitated at certain time intervals after injection of the substances. Mast cells were studied in film preparations of serous membranes from the mesentery of the small intestine, omentum, pericardium, and renal capsule, fixed in formalin solution buffered to pH 7.4, and stained with 0.1% toluidine blue solution, pH 4.9. Morphometric criteria were obtained by counting more than 800 mast cells from each animal. The combined morphometric method [2] included: preparing cytograms for four categories of cells, based on the number of granules and degree of metachromasia; 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, allowing for the relative frequency of its weak, moderate, and

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TABLE 1. Changes in Total Fibrinolytic Activity (I), Nonenzymic Fibrinolysis (II) (in mm^2), and Thrombin Time (III) (in sec) after Intravenous Injection of 0.85% NaCl Solution, α-Thrombin, and Trypsin into Rats (M ± m)

Parameter studied	Time after injection of substance, min	Substance		
		0.85 % NaCl	α-Thrombin	trypsin
I	1	41,2±0,7 (30)	45,4±0,7 (23)	45,7±0,7 (11)
	5	43,8±0,5 (30)	58,1±0,8 (23)*	51,0±2,7 (11)
	30	43,2±0,4 (30)	43,1±0,9 (22)	42,3±1,7 (10)
	120	42,2±0,6 (30)	44,8±0,7 (22)	39,2±1,4 (10)
II	1	35,1±0,6 (30)	35,5±0,7 (23)	35,0±0,6 (11)
	5	35,6±0,5 (30)	48,0±1,0 (23)*	36,6±1,0 (11)
	30	33,7±0,7 (30)	36,6±1,0 (17)	34,6±1,2 (11)
	120	34,8±0,4 (30)	36,8±0,4 (17)	38,0±1,1 (11)
III	1	26,4±0,2 (23)	26,4±0,3 (20)	25,8±0,5 (14)
	5	26,1±0,3 (23)	34,4±0,8 (32)*	25,6±0,2 (14)
	30	26,0±0,2 (24)	26,0±0,4 (28)	25,5±0,3 (11)
	120	25,2±0,2 (22)	26,6±0,5 (10)	25,1±0,3 (11)

Legend. Here and in Table 2 number of animals shown in parentheses; *P < 0.001.

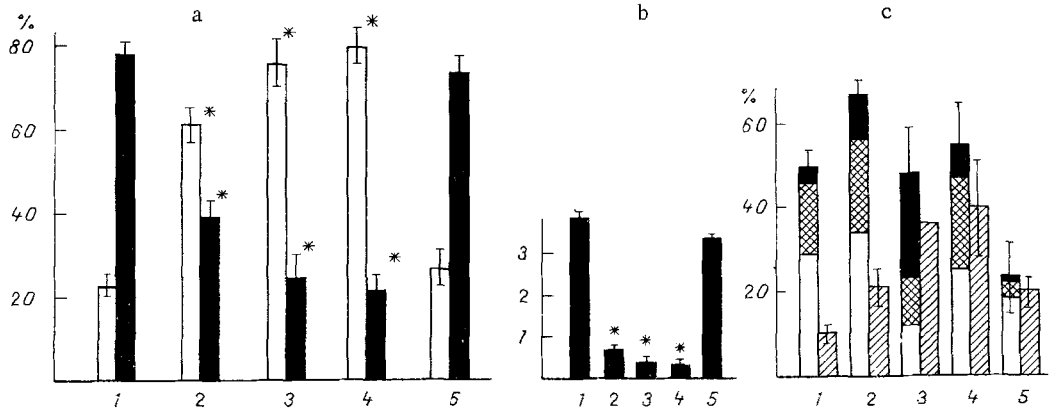

Fig. 1. Dynamics of morphometric criteria of state of mast cell population in rats 1 min (2), 5 min (3), 30 min (4), and 120 min (5) after injection of α-thrombin. 1) Intravenous injection of 0.85% NaCl solution: a) cytograms of mast cell population; relative frequency (in %) of all pale cells (unshaded columns) and of all dark cells (shaded columns); b) heparin saturation index of cells; c) degranulation index and relative frequency of its forms (columns on left); unshaded part of columns denotes weak form, line-shaded part denotes moderate, and part-shaded black denotes strong form of degranulation; index of granulolysis (columns on right); *P < 0.001.

strong forms — the ratio of the total number of degranulated cells to the total number of cells; the granulolysis index also was calculated as the ratio of the total number of emptied, very pale cells to the total number of cells, and the overall secretion index — obtained by adding the indices of degranulation and granulolysis, also were calculated. The numerical results were subjected to statistical analysis by computer.

EXPERIMENTAL RESULTS

The data in Table 1 on the dynamics of heparin secretion during activation of the anticlotting system by α-thrombin show that levels of nonenzymic fibrinolysis, reflecting the content of complex compounds of heparin, and also of total fibrinolytic activity were significantly raised by the 5th minute of the experiment compared with the control. At the 30th minute of the experiment the values of these parameters were lower. By the 120th minute of the experiment, activity of heparin complexes no longer differed from the control. Thrombin time, reflecting plasma anticoagulant activity, correlated with a change in activity of heparin complexes and also was significantly increased at the 5th minute of the experiment (Table 1).

TABLE 2. Effect of α-Thrombin and Trypsin on State of Mast Cell Population after Intravenous Injection of Substances (M ± m)

Morphometric criterion of state of mast cell population	Substance		
	0,85 % NaCl	α-thrombin	trypsin
Total number of dark cells	77,1±1,8 (16)	24,1±6,1 (13)*	71,2±4,6 (10)
Total number of pale cells	22,9±1,8 (16)	75,9±6,1 (13)*	25,8±5,4 (10)
Heparin saturation index of cells	3,9±0,6 (16)	0,35±0,1 (13)*	2,75±1,0 (10)
Degranulation index	50,0±3,6 (16)	49,0±15,7 (13)	49,1±6,6 (10)
Granulolysis index	10,4±2,0 (16)	36,1±10,6 (13)	13,0±4,1 (10)
Overall index of secretion	60,5±2,8 (16)	84,3±6,3 (13)	62,1±4,4 (10)

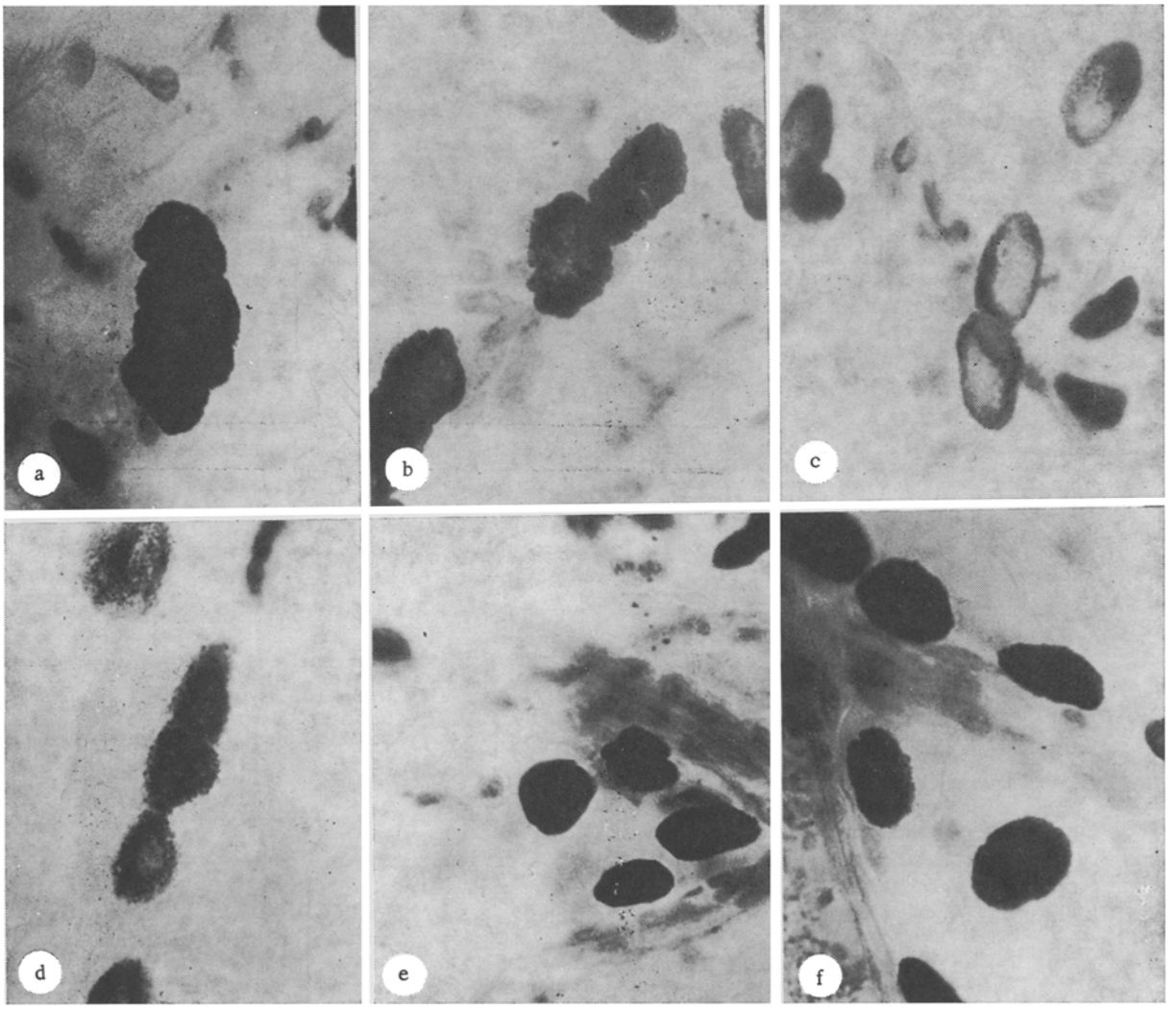

Fig. 2. Changes in mast cell population of rats after injection of α-thrombin (b, c, d, f) and trypsin (e). a) Control injection of 0.85% NaCl; b) 1 min, c) 5 min, d) 30 min, f) 120 min after injection of α-thrombin; e) 5 min after injection of trypsin. Stained with toluidine blue, 400×.

The results of the morphometric analysis of the state of the mast cell population in rats of this series, compared with control animals, are given in Fig. 1. Whereas in the control rats dark cells, filled with densely packed metachromatic granules (Fig. 1: 2a), with a high heparin saturation index, predominated, a significant shift of the mast cell population toward pale cells free from heparin was observed as early as 1 min after injection of α-thrombin (Fig. 1: 2b). The heparin saturation index of the cells fell below unity because of an increase in the number of pale and degranulated cells to 60.9%. By the 5th min-

ute of the experiment the number of these cells had increased to 75.9%, but the heparin saturation index of the cells had fallen to 0.35 (Fig. 1: 2c). During 30 min of the experiment, the number of mast cells free from heparin remained at a high level (Fig. 1: 2d). Not until after 120 min of the experiment were the morphometric criteria of the mast cell population restored to their basal level (Fig. 1: 2f).

The study of the mast cell population of rats after injection of α-thrombin thus indicates that heparin secretion as a result of effector activity of the anticlotting system takes place as early as at the first minute of the experiment. Secretion reached a maximum at the 5th minute of the experiment (Fig. 2c).

Intravenous injection of trypsin in a molar concentration corresponding to that of α-thrombin caused no significant changes in activity of heparin complex or in thrombin time within the period of the investigation (Table 1). The increase in total fibrinolytic activity at the 5th minute of the experiment was due to activation of plasminogen by trypsin. The absence of a response of activation of the anticlotting system after injection of trypsin also was confirmed by analysis of the state of the mast cell population at the 5th minute of the experiment (Table 2; Fig. 2e). It will be clear from Table 2 that the heparin saturation index of the cells and the overall index of secretion of the cells did not differ significantly from the corresponding control parameters. Meanwhile, after injection of α-thrombin the heparin saturation index of the cells fell by almost one order of magnitude (Table 2; Fig. 1b).

It can be concluded from these results and those obtained previously [6] that the high proteolytic and esterase activity of trypsin is insufficient for specific interaction of the enzyme with receptors of the vessel wall and to stimulate increased heparin secretion by mast cells. Analysis of the dynamics of heparin secretion after injection of α-thrombin shows that activity of the mast cell population correlates with excitation of the anticlotting system, as reflected in its effector activity, namely the appearance of heparin complexes in the blood. Intensification of heparin secretion by the mast cells evidently reflects the active state of the anticlotting system.

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