HEPARIN SECRETION BY PERITONEAL MAST CELLS STIMULATED BY α-THROMBIN IN RATS

B. A. Umarova, S. V. Khlgatyan, and S. M. Strukova

UDC 612.112.93.015.36:612.115.35].063:612.115.12

KEY WORDS: thrombin; heparin; mast cells

Synthesis, storage, and secretion of the vitally important anticoagulant heparin are functions of mast cells. Two phenotypes of cells are distinguished: connective-tissue and mucosal [9]. Connective-tissue mast cells contain heparin, histamine, and other biologically active substances. Instead of heparin, the mucosal mast cells contain chondroitin sulfate [7]. Both types of cells respond to the action of various inducers (monoamines, polyamines, antigens, ionophores, enzymes, etc.) by secreting the contents of their granules. Thrombin, a regulatory enzyme of blood clotting, may also be an inducer of secretion. However, it has been shown that thrombin does not activatve isolated mouse peritoneal mast cells [10].

The present writers found previously [12] that increased heparin secretion by mast cells is included in the effector act of the anticlotting system in response to its excitation by α-thrombin and its proteolytically inactive analogs. However, the mechanism of heparin secretion by mast cells remains unexplained. Thrombin does not penetrate into the interstitial space and it is not known how the external signal is transmitted to the mast cells from the appearance of the enzyme in the blood stream. Meanwhile the question of direct interaction between mast cells and thrombin is of particular interest in connection with the fact that connective tissue cells (fibroblasts), smooth-muscle cells, nerve tissue cells (neuroblasts), and blood cells (except erythrocytes) have receptors on their surface which specifically bind thrombin [8].

Hence the need to investigate interaction between thrombin and mast cells in a system in vitro. To reveal the character of thrombin reception by mast cells, whether it is connected with proteolysis of the receptor or with interaction of agonist – receptor type, a possible approach is to use an α-thrombin analog in whose structure a particular region of the enzyme molecule (either the active center or the recognition center for high-molecular-weight compounds) has been selectively modified.

The aim of this investigation was to study the possibility that heparin is secreted by rat peritoneal mast cells under the influence of α-thrombin and its analogs [β/γ-thrombin, diisopropyl phosphate (DIP)-α-thrombin].

EXPERIMENTAL METHOD

Mast cells were obtained from the peritoneal fluid of rats by the method in [12]. The animals were anesthetized, decapitated, and exsanguinated. An intraperitoneal injection of 67 mM Na-phosphate buffer, pH 7.0, containing 145 mM NaCl was given. Peritoneal fluid was collected 2 min later. Mast cells were isolated in a Ficoll-400 ("Pharmacia") density gradient from 30 to 40%, and washed 3 times with a balanced solution (67 mM Na-phosphate buffer, pH 7.0, containing 145 NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 1 mg/ml albumin, and 1.8 mg/ml glucose). The washed cells were counted in a Goryaev counting chamber, in a dilution of 100 times.

The cell pool thus obtained contained 80-85% of mast cells. The viability of the cells was judged by their reaction to staining with vital dye (1% trypan blue solution).

In the experiments $4 \cdot 10^6$ viable cells in a volume of 100 μliters were incubated with an equal volume of ligand for 5 min at 37°C. After centrifugation, heparin [6] and histamine [11] were determined in the supernatant. The ligands used were α-thrombin, purified by the method in [3], with clotting activity of 3500 NIH units/mg protein in concentrations of between 10^{-11} and 10^{-6} M, β/γ-thrombin, obtained by T. N. Dugina by the method in [4], with esterase activity of 4 μmoles BAME/mg protein/min, in concentrations of 10^{-9} to 10^{-6} M, and

M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 131-133, February, 1989. Original article submitted March 5, 1988.

0007-4888/89/0002-0133$12.50

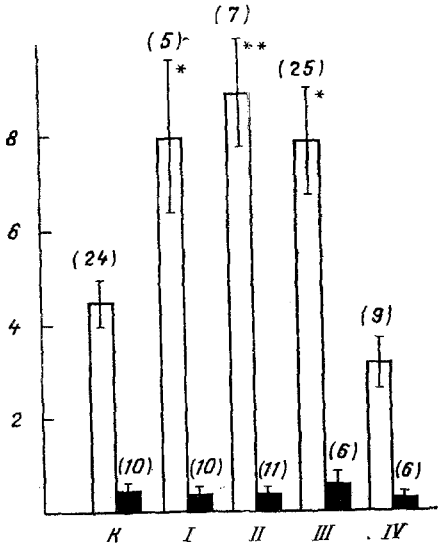

Fig. 1

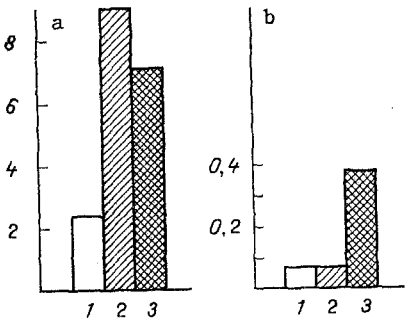

Fig. 2

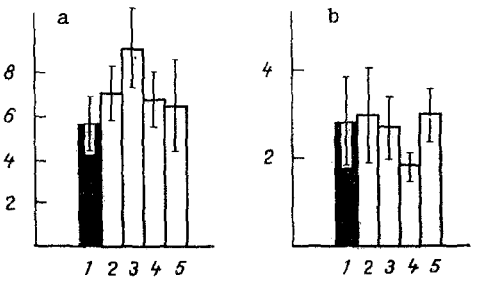

Fig. 3

Fig. 1. Secretion of heparin (unshaded columns) and histamine (black columns, in $\mu g/4 \cdot 10^6$ cells, ordinate) by rat peritoneal mast cells under the influence of various concentrations of α-thrombin. Abscissa, concentration of α-thrombin: I) 10^{-11} M; II) 10^{-9} M; III) 10^{-8} M; IV) 10^{-6} M. Number of experiments shown in parentheses. K) Control. *$p < 0.01$, **$p < 0.001$.

Fig. 2. Secretion of heparin (a) and histamine (b; in $\mu g/4 \cdot 10^6$ cells) by rat peritoneal mast cells under the influence of 10^{-9} M α-thrombin (2) and polymyxin M in a concentration of 3 μg/ml (3). 1) 0.85% NaCl.

Fig. 3. Heparin secretion (in $\mu g/4 \cdot 10^6$ cells) by rat peritoneal mast cells in response to the action of β/γ-thrombin (a) and of DIP-α-thrombin (b). In a: 1) 0.85% NaCl; 2-5) 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M β/γ-thrombin; in b: 1) 0.85% NaCl; 2-5) 10^{-12}, 10^{-10}, 10^{-9}, and 10^{-8} M DIP-α-thrombin.

DIF-α-thrombin with residual clotting activity of 0.015 NIH unit/mg protein in concentrations of between 10^{-12} and 10^{-8} M, and polymyxin M (3 μg/ml).

EXPERIMENTAL RESULTS

The results of experiments to study the effect of α-thrombin on secretion of heparin and histamine by peritoneal mast cells are illustrated in Fig. 1. With α-thrombin in concentrations of 10^{-11} to 10^{-6} M no increase in histamine secretion by the mast cells was found compared with control samples. Meanwhile α-thrombin in a concentration of 10^{-11} M increased heparin secretion by 75.5% compared with the basal level. With the enzyme in a concentration of 10^{-9} M the quantity of heparin secreted was twice the initial level. The effective concentration of α-thrombin (10^{-11} M), incidentally is much lower than the possible physiological concentration of the enzyme [5].

It was shown previously that mouse peritoneal mast cells do not secrete histamine and β-hexosaminidase [10]. The present results confirm the absence of increased histamine secretion by rat mast cells in response to the action of thrombin. However, under these same experimental conditions a significant increase in heparin release from the mast cells was observed. Comparative analysis of data on heparin and histamine secretion indicates selective secretion of heparin in response to stimulation of the mast cells by α-thrombin. This hypothesis is supported by the results of the experiments shown in Fig. 2. Polymyxin M, a well-known histamine liberator, induces (in a concentration of 3 μg/ml) an increase in secretion of heparin (4.1 $\mu g/4 \cdot 10^6$ cells) and of histamine (0.33 $\mu g/4 \cdot 10^6$ cells). In the same series of experiments α-thrombin in a concentration of 10^{-9} M induced only release of heparin (6.7 $\mu g/4 \cdot 10^6$ cells), and under these circumstances histamine secretion was the same as the control level.

In the next series of experiments the effect on heparin secretion of α-thrombin analogs, namely β/γ-thrombin, in which the catalytic center of the enzyme is preserved but the recognition site for high-molecular-weight compounds is destroyed, and DIP-α-thrombin, a proteolytically inactive form of the enzyme, in which the serine of the active center is alkylated, was investigated. It will be clear from Fig. 3 that β/γ-thrombin in a concentration of 10^{-8} M did not lead to any significant increase in heparin secretion. This concentration of β/γ-thrombin is 2 or 3 orders of magnitude higher than the effective concentration of α-thrombin

DIP-α-thrombin (Fig. 3b), in the range of concentrations studied (10^{-12}-10^{-8} M) did not stimulate the mast cells and the level of heparin secreted did not exceed the control.

Thus analysis of interaction of α-thrombin and its analogs with rat peritoneal mast cells showed that α-thrombin stimulates heparin secretion provided that the enzyme molecule contains both its catalytic center and its recognition center for high-molecular-weight compounds. The low effective concentration of the ligand suggests that specific receptors for thrombin, controlling the mechanism of secretion, exist on the surface of the mast cells.

LITERATURE CITED

1. B. A. Kudryashova, B. A. Umarova, S. M. Strukova, et al., Fiziol. Zh. SSSR, No. 10, 1460 (1980).
2. A. I. Strukov, S. M. Strukova, T. G. Khlebnikova, et al., Byull. Éksp. Biol. Med., No. 6, 116 (1982).
3. S. M. Strukova, B. A. Umarova, E. G. Kireeva, et al., Biokhimiya, No. 4, 708 (1978).
4. S. M. Strukova, B. A. Umarova, and O. A. Semenova, Fiziol. Zh. SSSR, 65, No. 5, 702 (1975).
5. D. L. Aronson, L. Stevan, A. P. Ball, et al., J. Clin. Invest., 60, 1410 (1977).
6. L. Enerback, J. Histochem., 42, 301 (1974).
7. L. Enerback, Mast Cell Differentiation and Heterogeneity, ed. by A. D. Befus et al., New York (1986), pp. 1-26.
8. J. W. Fenton II, Cell Proliferation: Recent Advances, ed. by A. L. Boynton and H. L. Leffert, Vol. 2, New York (1987), p. 4.
9. J. Kitamura, T. Nakano, and J. Kanakura, Dev. Growth Diff., 28, 321 (1986).
10. E. Razin, D. Baranes, and G. Marx, Exp. Cell Res., 160, 380 (1985).
11. P. A. Shore, A. A. Burchalter, and V. H. Cohn, J. Pharmacol. Exp. Ther., 127, 182 (1959).
12. B. Uvnas, Acta Physiol. Scand., 71, 303 (1967).

CHARACTERISTICS OF PROTEOLYSIS IN THE GASTROINTESTINAL TRACT IN THE EARLY POSTNATAL PERIOD

V. R. Nikolaevskaya

UDC 612.322.4+612.332.4]:612.648

KEY WORDS: artificial feeding; casein; glycomacropeptide

An important problem at this time is the study of proteolysis of experimental samples of casein in the neonatal gastrointestinal tract with a view to creating substitutes for human milk that will satisfy the physiological demands of the neonatal period. Experimental results have shown the functional immaturity of the proteolytic system for protein digestion in the stomach and the considerable maturity of the proteolytic system for protein digestion in the small intestine [1, 2]. The combination of these effects leads to a shift of the peak of protein digestion in the period of milk feeding toward the distal portion of the small intestine.

The aim of this investigation was to study the specific nature of hydrolysis and assimilation of an "experimental" sample of casein, purified from glycomacropeptide.

EXPERIMENTAL METHOD

Experiments were carried out on 90 Wistar rats transferred from the 15th to the 21st day to artificial feeding on a milk substitute, with a control or experimental sample of casein as the protein component. The fractional composition of the chyme was studied by gel-chromatography with Sephadex G-75 [2]. Protein was determined by Lowry's method [6]. Concentrations of amino acids in the experimental and control samples of casein were determined jointly

Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 133-135, February, 1989. Original article submitted January 22, 1988.