Effect of Activated Protein C on Secretory Activity of Rat Peritoneal Mast Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 10, pp. 382-385, October, 2006
Original article submitted March 7, 2006

Generation of thrombin and activated protein C in the inflammatory focus was demonstrated in rats with experimental acute peritonitis. The contents of thrombin and activated protein C peaked by the 30th and 120th minute of inflammation, respectively. *In vitro* study showed a decrease in spontaneous and compound 48/80-induced secretion of β-hexosaminidase by peritoneal mast cells under the influence of activated protein C in low concentrations. The antiinflammatory effect of protein C in the focus of acute peritonitis is probably realized through NO release from peritoneal mast cells. This conclusion is derived from the data that L-NAME abolishes the protective effect of activated protein C.

Key Words: *mast cells; inflammation; activated protein C; thrombin; protein kinase-activated receptors*

Activation of blood coagulation during inflammation leads to the appearance of serine proteases (factors VIIa, Xa, and thrombin). These compounds activate blood and connective tissue cells and play a role in provocation and regulation of inflammation. Newly formed thrombin interacts with the receptor (endothelial thrombomodulin) and converts the proenzyme (protein C) into serine proteinase (activated protein C, APC). APC cleaves active forms of blood coagulation factors V and VII thus blocking thrombin formation. Apart from anticoagulant activity, APC exhibits antiinflammatory and antiapoptotic properties [5,14].

The effect of thrombin on cells is realized via the interaction with some membrane receptors, including proteinase-activated receptors (PAR) [9,10, 12]. Thrombin in low concentration, as well as APC in high concentration, can activate PAR1 [6,10]. These receptors are expressed on cells involved in blood coagulation, inflammation, and tissue reparation. PAR-expressing mast cells are also involved in the inflammatory process [3]. Our studies showed that the response of peritoneal mast cells (PMC) to thrombin increases in rats with experimental acute peritonitis, which reflects activation of PAR1 expression under these conditions [4] and suggests generation of thrombin and other proteinases of the blood coagulation system in the inflammatory focus. Thrombin converts blood protein C into APC. There is no evidence that these proteinases appear in the peritoneal cavity during acute inflammation. The effect of APC on functional activity of mast cells remains unknown.

Here we measured the contents of thrombin and APC in the inflammatory focus of rats with experimental acute peritonitis. The effect of APC on secretory activity of rat PMC was studied *in vitro*.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g.

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Acute peritonitis in rats was induced by intraperitoneal injection of sodium thioglycollate (Fluka) in a dose of 4 g/kg [8]. The animals were decapitated 1, 30, 60, 120, 180, and 300 min after the induction of peritonitis. Peritoneal lavage fluid was obtained. The cells were separated by centrifugation. Total protein content [2] and activities of thrombin and APC were measured in 3 parallel samples of the supernatant. To estimate amidolytic activity of enzymes, the samples containing 5 μg total protein were incubated with Tris-HCl (pH 8.4), 150 μM chromogenic thrombin-specific substrate S-2238 (N-p-Tosyl-Gly-Pro-Arg-*p*-nitroanilide, Chromogenix), or APC-specific S-2366 (pyroGlu-Pro-Arg-*p*-nitroanilide, Chromogenix) at 37°C. Amidolytic activity of enzymes was estimated spectrophotometrically by the release of *p*-nitroanilide at 405 nm. PMC were isolated from the peritoneal fluid [13]. Activity of PMC was determined by the release of β-hexosaminidase [11]. The enzyme and inflammatory mediator histamine are secreted by PMC [7]. PMC were incubated in the presence of APC (37°C, 10 min) and treated with liberating agent compound 48/80 or HEPES/NaCl buffer (10 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 0.1% bovine serum albumin; pH 7.4). β-Hexosaminidase activity was estimated by disintegration of a specific chromogenic substrate *p*-nitrophenyl-N-acetyl-β-D-glucosaminide (Sigma) and calculated as follows:

$$A/(A+B)\times 100\%,$$

where A and B are the contents of mediator in the supernatant and precipitate, respectively.

The results were analyzed by Student's *t* test for paired samples.

RESULTS

In series I, we measured the contents of thrombin and APC in the peritoneal cavity of rats with acute inflammation. Thrombin was rapidly generated in the peritoneal cavity, the peak concentration was attained by the 30th minute of acute inflammation (1.38±0.35 nmol/mg protein; Fig. 1, *a*). By the 60th minute, APC concentration in the peritoneal cavity 3.6-fold exceeded the basal level (1st minute of inflammation). This parameter peaked 120 min after induction of inflammation (4.91±0.91 nmol/mg protein; Fig. 1, *b*).

The dynamics of vascular permeability in the small intestine was studied using Evans blue dye. Vascular permeability sharply increased 30 min after the start of the study (by 2 times), which is typical of the exudation stage [4].

In vitro study was performed to estimate the effect of APC on secretory activity of PMC under normal conditions and during activation with liberating agent compound 48/80 (Sigma). APC in concentrations of 0.5 and 1.0 nM significantly decreased β-hexosaminidase secretion (5.9 and 9.3%, respectively, $p<0.05$) compared to spontaneous level (13.0%, Fig. 2). Spontaneous secretion decreased insignificantly under the influence of APC in higher concentrations (2.5-3.0 nM). The decrease in β-hexosaminidase secretion is probably related to the release of intracellular nitric oxide (NO), which inhibits secretion of inflammatory mediators [1]. To test this hypothesis, PMC were preincubated with a NO synthesis inhibitor L-NAME (300 μM, 30 min). β-Hexosaminidase secretion in the presence of L-NAME was significantly higher compared to APC-induced secretion ($p<0.01$). Pretreatment with L-NAME had no effect on spontaneous secretion (Fig. 2).

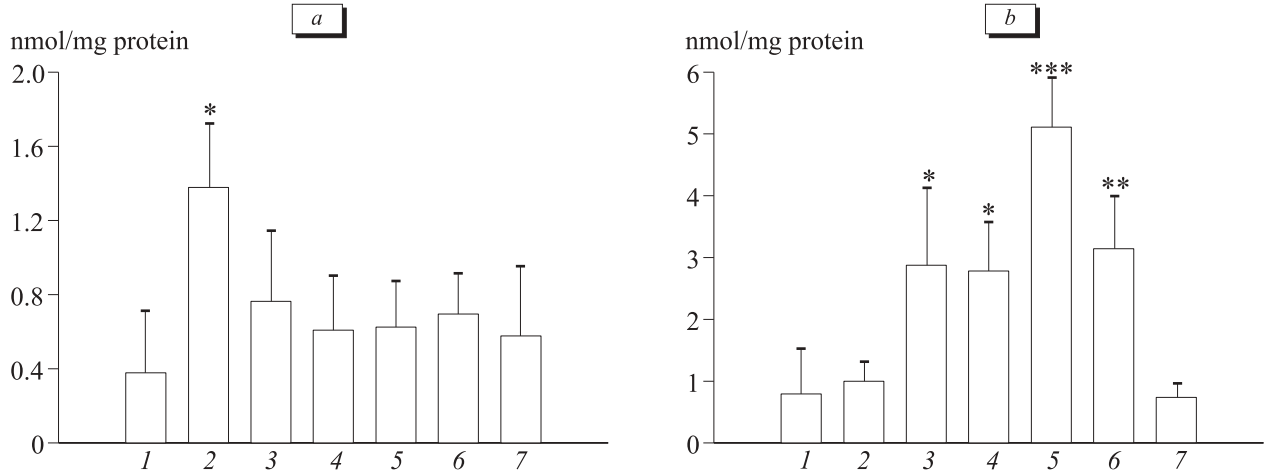

Fig. 1. Generation of thrombin (*a*) and APC (*b*) in the peritoneal cavity of rats 1 (*1*), 30 (*2*), 60 (*3*), 90 (*4*), 120 (*5*), 180 (*6*), and 300 min (*7*) after the induction of acute inflammation ($n=5$). *$p<0.05$, **$p<0.01$, and ***$p<0.005$ compared to the 1st minute of inflammation.

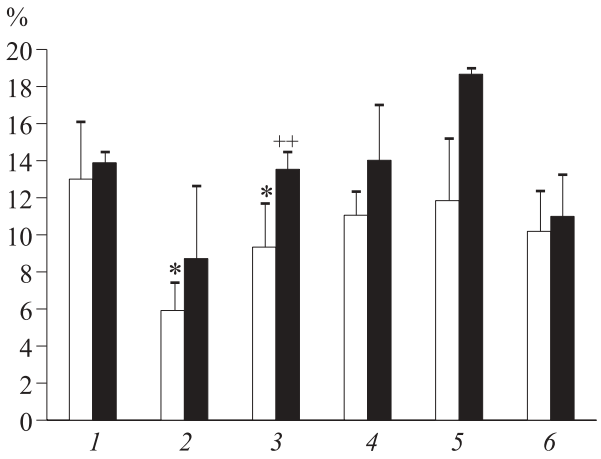

Fig. 2. β-Hexosaminidase secretion by rat PMC under the influence of APC (0.5-5.0 nM). Effect of 30-min preincubation of PMC with L-NAME on β-hexosaminidase secretion. Spontaneous secretion of β-hexosaminidase by PMC (control, *1*); secretion under the influence of APC in concentrations of 0.5 (*2*), 1 (*3*), 2.35 (*4*), 3 (*5*), and 5 nM (*6*). Here and in Fig. 3: light bars, without L-NAME; dark bars, in the presence of L-NAME. $^{*}p<0.05$ compared to the control; $^{+}p<0.01$ compared to 1 nM APC without L-NAME. Here and in Fig. 3: results of 4 experiments.

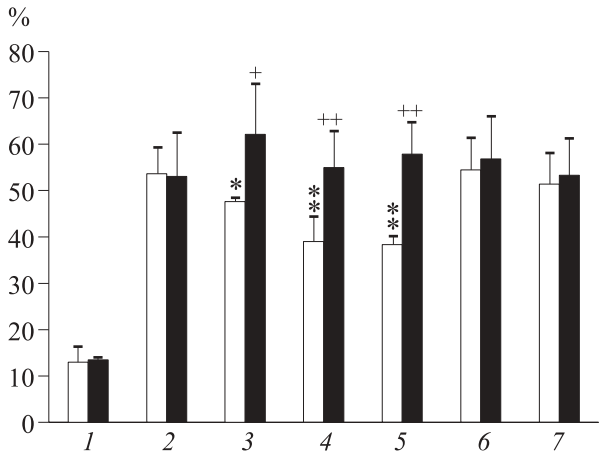

Fig. 3. Compound 48/80-induced secretion of β-hexosaminidase by PMC under the influence of APC (0.5-5.0 nM). Effect of 30-min preincubation with 300 μM L-NAME on β-hexosaminidase secretion (*1-7*). Spontaneous activity of PMC (*1*); 400 μg/ml compound 48/80 (*2*); preincubation of PMC with APC in concentrations of 0.5 (*3*), 1 (*4*), 2.35 (*5*), 3 (*6*), and 5 nM (*7*). $^{*}p<0.05$ and $^{**}p<0.01$ compared to compound 48/80-induced secretion; $^{+}p<0.05$ and $^{++}p<0.01$ compared to compound 48/80-induced secretion in the presence of 0.50, 1.00, and 2.35 nM APC without L-NAME.

In series II, the effect of APC on PMC activated with compound 48/80 (400 μg/ml) was studied by measuring the release of β-hexosaminidase. APC in concentrations of 0.5-2.3 nM significantly decreased β-hexosaminidase secretion by PMC induced by compound 48/80 ($p<0.01$, Fig. 3). APC in higher concentrations (3 and 5 nM) had little effect on induced secretion of β-hexosaminidase (Fig. 3). An APC-induced decrease in mediator secretion by PMC treated with compound 48/80 is probably associated with stimulation of NO release. NO inhibits induced secretion of mediators by mast cells (including β-hexosaminidase). To test this hypothesis, mast cells were pretreated with NO synthase blocker L-NAME (300 μM) before agonist administration. β-Hexosaminidase secretion that decreased under the influence of APC returned to high level after 30-min preincubation of PMC with L-NAME. Therefore, NO plays a role in the antiinflammatory effect of enzyme on mast cells.

Our results suggest that an antiinflammatory effect of APC in the focus of acute inflammation is related to the regulation of mast cell activity.

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