Secretory Activity of Mast Cell during Stress: Effect of Prolyl-Glycyl-Proline and Semax

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Stress increased secretory activity of mast cells in the mesentery and subcutaneous fat of rats. Intraperitoneal injection of Semax and prolyl-glycyl-proline in doses of 0.05 and 1 mg/kg, respectively, 1 h before stress abolished this effect. The test preparations did not modulate secretory activity of mast cells in unstressed animals. Semax and prolyl-glycyl-proline *in vitro* prevented activation of mast cells with synacten and acetylcholine. The stabilizing effect of peptides on mast cells probably determines their antiulcer activity.

Key Words: *stress; mast cells; proline-containing peptides*

Stress plays an important role in the pathogenesis of peptic ulcer disease. Stress factors sharply increase secretory activity of mast cells (MC) and production of proinflammatory factors inducing damage to the gastric mucosa [7,8,10]. Substances stabilizing MC reduce the number of gastric mucosa lesions and are used in medical practice as antiulcer preparations [8,9].

Studies of ulceration in rats showed that short proline-containing peptides protect the gastric mucosa from injury [1-3,12,13]. The tripeptide prolyl-glycyl-proline (PGP) and $ACTH_{4-7}$ analogue Semax with C-terminal Pro-Gly-Pro sequence produced a strong protective effect during ulceration under conditions of water-immersion stress.

Taking into account the important role of MC in ulceration, we hypothesized that the protective effect of proline-containing peptides is partially due to their influence on secretory activity of these cells. The effect of proline-containing peptides on MC remains unknown. Here we studied the effects of PGP and Semax on secretory activity of MC on various models of stress in rats.

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MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 200-280 g.

We used 3 models of stress: immobilization stress (1-h fixation on a table), water-immersion stress (3-h immobilization on the back to a plate and vertical immersion in water up to the level of the diaphragm at 16°C), and intraperitoneal injection of cholecystokinin-4 (CCK-4, 100 μg/kg) [5].

The rats were deprived of food for 24 h before the experiment, but had free access to water. The test peptides were administered 1 h before stress. The animals received Semax and PGP in doses of 0.05 and 1 mg/kg, respectively. The rats were decapitated after stress. Samples of the mesentery and subcutaneous fat were taken for evaluation of secretory activity of MC. Film preparations were fixed in 0.1% formalin and stained with 0.1% toluidine blue. Functional state of MC was analyzed by light microscopy. We calculated the ratio between the numbers of light depleted cells (index of granulolysis) and degranulated cells (index of degranulation) and total count of cells. The degree of degranulation (low, medium, and high) depended on the amount of granules released from cells [5].

We studied *in vitro* effects of peptides on spontaneous degranulation of MC. Samples of the mesentery

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and subcutaneous fat from intact rats were incubated with PGP (6×10^{-5} M) or Semax (1.2×10^{-6} M) for 15 min. To evaluate the effects of PGP and Semax on induced degranulation tissue samples were pretreated with peptides and incubated with secretion-stimulating agents substance 48/80 (2×10^{-5} g/ml), acetylcholine (1×10^{-5} g/ml), and $ACTH_{1-24}$ analogue synacten (2×10^{-5} M). Control experiments were performed with physiological saline.

The significance of differences was evaluated by means of ANOVA (STATISTICA software).

RESULTS

Stress activated MC in the mesentery and subcutaneous fat of rats. We observed not only an increase in the number of degranulated cells (index of degranulation), but also a rise in the count of cells undergoing medium and pronounced degranulation (Fig. 1). The changes were most pronounced after 3-h water-immersion stress. The index of degranulation and the number of highly degranulated cells increased 2.2- and 9-fold, respectively. One-hour immobilization produced a less pronounced effect. Under these conditions the index of degranulation and the number of highly degranulated cells increased by 1.5 and 4 times, respectively. One hour after injection of CCK-4 the index of degranulation and the number of highly degranulated cells increased by 1.8-fold and 3.9-fold, respectively. Our results confirm the data that MC are involved in the general adaptive reaction to stress [6,8,10].

The release of mediators from MC during stress is realized via granulolysis and degranulation. We previously studied changes in secretory activity of MC during immobilization stress [6] and administration of CCK-4 [5]. The mechanism of secretion depends on the type, strength, and duration of stress. Granulolysis is a physiological mechanism of secretion that plays a major role during moderate stress. Our experiments showed that the contribution of degranulation increased with increasing the strength of stress. After severe 3-h water-immersion stress the index of degranulation sharply increased, while the index of granulolysis was below the initial level.

Intraperitoneal injection of Semax and PGP (0.05 and 1 mg/kg, respectively) before stress prevented the increase in secretory activity of MC. The index of degranulation increased insignificantly. The count of MC with various degrees of degranulation remained practically unchanged (Fig. 1). It should be emphasized that the test peptides had no effect on stress-produced changes in granulolysis.

Intraperitoneal injection of peptides did not modulate functional activity of MC in unstressed rats.

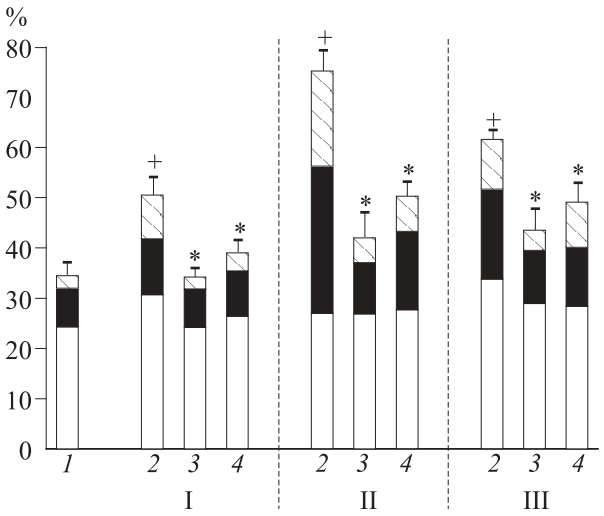

Fig. 1. Degranulation of mast cells in the mesentery and subcutaneous fat on various models of stress after treatment with physiological saline (*2*), Semax (*3*), or PGP (*4*). Control (*1*), immobilization (*I*), water-immersion stress (*II*), and cholecystokinin-4 administration (*III*). Here and in Fig. 2: light bars — insignificant degranulation. Dark bars: intermediate degranulation. Shaded bars: pronounced degranulation. Combined results of experiments with the mesentery and subcutaneous fat. $^{+}p<0.01$ compared to group 1; $^{*}p<0.01$ compared to group 2.

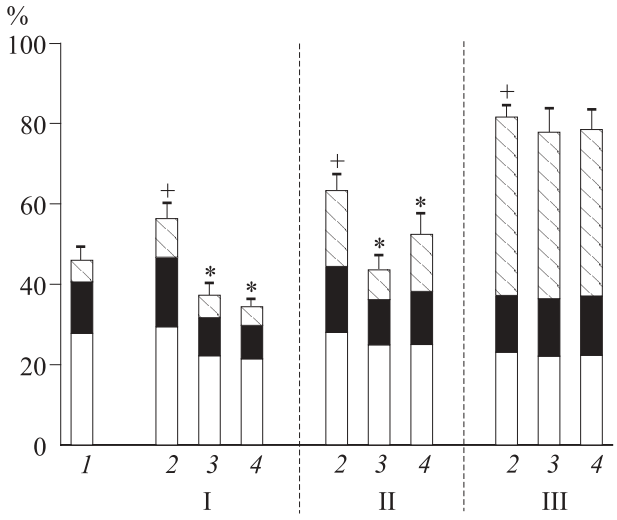

Fig. 2. Degranulation of mast cells during *in vitro* activation with synacten, acetylcholine, and substance 48/80 after preincubation with physiological saline (*2*), Semax (*3*), or PGP (*4*). Control (without activators, *1*), synacten (*I*), acetylcholine (*II*), and substance 48/80 (*III*).

Our results show that Semax and PGP blocked degranulation of MC during stress, but had no effect on spontaneous secretion. We hypothesized that the inhibitory effect of Semax and PGP (similarly to amylin [4]) on secretory activity of MC can be related to the direct stabilizing influence of peptides. In the next experimental series we studied *in vitro* effects of Semax and PGP on the secretory response of MC to activators (Fig. 2). Activation of MC with synacten, acetylcholine, and substance 48/80 increased the index

of degranulation and count of highly degranulated cells. Preincubation of tissue samples with Semax or PGP abolished the activating effect of synacten and acetylcholine on MC. However, pretreatment of MC with peptides had no effect on the secretory response to substance 48/80. These differences are probably associated with different mechanisms of cell activation. The influence of acetylcholine, ACTH, and its analogues and fragments is realized via receptors on the cell membrane. Activation of MC with the classic polybasic liberator substance 48/80 is mediated by another mechanism: the hydrophobic molecule of this substance is integrated into the cell membrane and directly binds to G-proteins [11].

The ability of PGP and Semax to prevent activation of MC with the $ACTH_{1-24}$ analogue synacten suggests that these peptides modulate a variety of reactions realized via ACTH. Taking into account similar effects of the peptides, we can hypothesize that the stabilizing effect of Semax on MC is partially due to the presence of the C-terminal sequence PGP.

The inhibitory effect of PGP and Semax on activation of MC probably contributes to the protective antiulcer influence of proline-containing peptides.

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