Peptide Correction of Disorders in Rat Mesenteric Microcirculatory System during Inflammation

B. A. Umarova, G. N. Kopylova, T. V. Lelekova, Z. V. Bakaeva, E. L. Goncharova, and G. E. Samonina

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PGP peptide had a protective effect in contractile dysfunction of the rat mesenteric lymph vessels under conditions of inflammation, irrespective of the time of its injection (before or after inflammatory agent). The preventive effect of this peptide is largely determined by its capacity to prevent mast cells activation. PGP injected 2 h after induction of inflammation did not inhibit secretory activity of mast cells, which suggests other mechanisms of its therapeutic action.

Key Words: *inflammation; lymph vessels; peptides; mast cells*

The development of acute peritonitis induced in rats by intraperitoneal injection of thioglycolate is paralleled by inhibition of the contractile activity of mesenteric lymph vessels and disorders in their reaction to norepinephrine (NE). Proline-containing peptides, injected directly after inflammation induction, exhibited a significant protective effect on the mesenteric microcirculatory system [4]. However, it remains unclear, during what stage of inflammation the effect of glyprolines is most pronounced and whether they can be used for preventive purposes. The mechanisms underlying the effect of glyproline also remain unclear. We hypothesized that correction of mesenteric microcirculatory dysfunction with glyproline in rats with experimental peritonitis was largely due to their stabilizing effect on mast cells [3], whose mediators modulated vascular permeability and were responsible for tissue edema during inflammation [6,10].

Prolyl-glycyl-proline (PGP) tripeptide exhibited the most pronounced effect on the microcirculatory system, and hence, the aim of this study was to clear out the therapeutic and preventive effects of PGP in disorders of the mesenteric lymph system in rats and involvement of mast cells in correction of these disorders with the peptide.

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** bellaum@mail.ru. B. A. Umarova

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (150-180 g) in accordance with the standards recommended by the European Scientific Foundation (ESF).

Peritonitis was induced in rats by intraperitoneal injection of 40% thioglycolate (Fluka) in a dose of 2 ml/200 g [9]. The mesenteric microcirculatory system was studied by vital microscopy 2 h after injection of thioglycolate by the method described previously [2].

The appearance of the mesentery was evaluated, the volume of exudation in the abdominal cavity was measured, and the reaction of lymph vessels to application of NE (10^{-6} M) was studied: the latency (from NE application to the start of contractions), number of contractions per minute, and duration of the period of contractions were recorded. The amplitude of contractions was measured on a monitor (in percent of the initial diameter of the vessel). The percent of vessels not responding to NE was evaluated.

The degree of mast cell degranulation was evaluated on film preparations of the mesentery as described previously [5].

The PGP tripeptide (synthesized at Institute of Molecular Genetics, Russian Academy of Sciences) was injected intraperitoneally in a dose of 3.7 μmol/kg.

Step I of the study was to evaluate the preventive and therapeutic effects of PGP in dysfunction of rat mesenteric lymph vessels caused by inflammation. Animals of experimental group 1 received the peptide 15 min before thioglycolate and were sacrificed 2 h after the development of peritonitis. Rats of experimental group 2 received the peptide 2 h after thioglycolate injection and were sacrificed 15 min after injection of the peptide. Controls were injected with saline during the same periods. The results in control groups were the same, and thereore the data from one of control groups are presented.

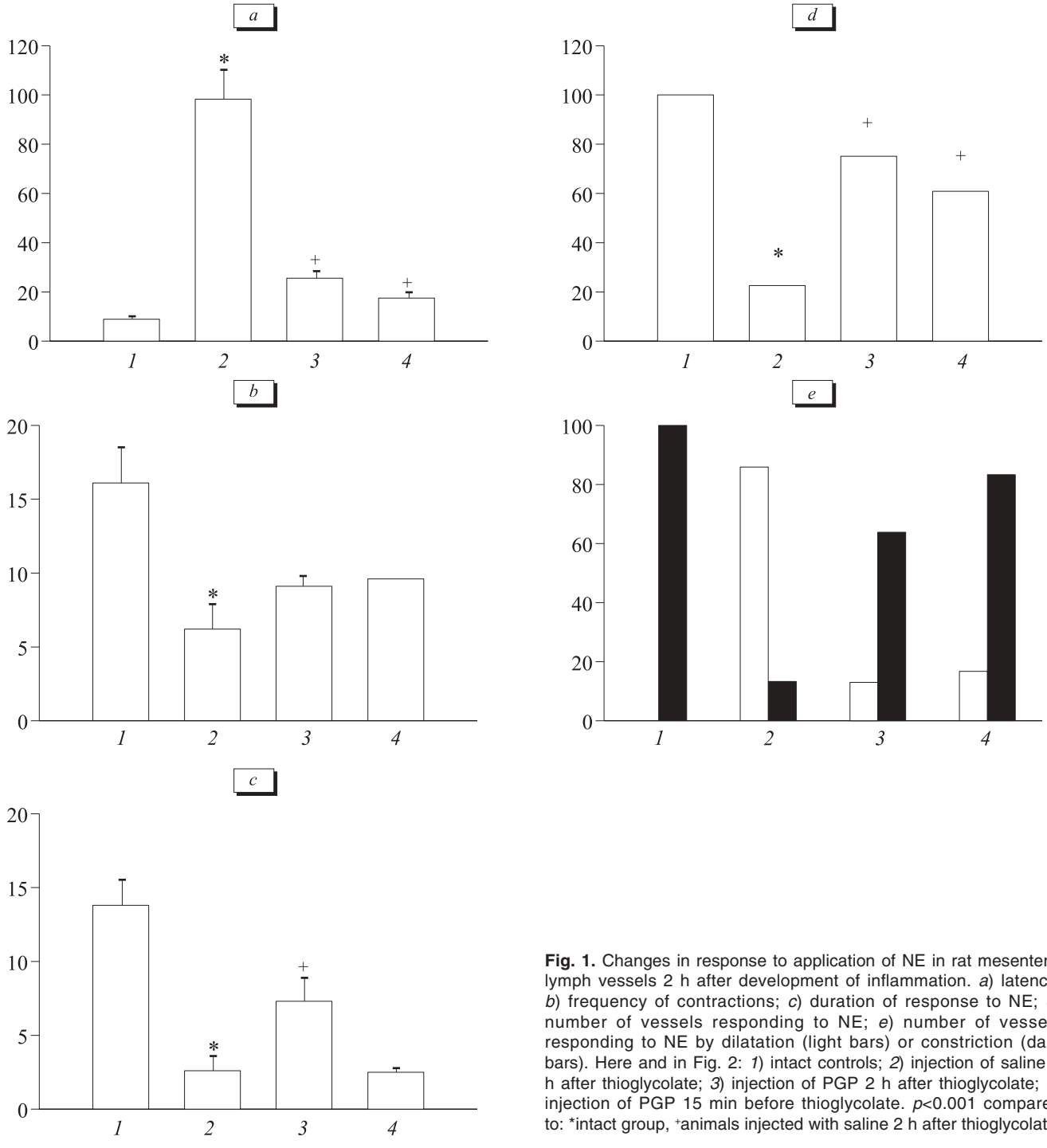

Fig. 1. Changes in response to application of NE in rat mesenteric lymph vessels 2 h after development of inflammation. *a*) latency; *b*) frequency of contractions; *c*) duration of response to NE; *d*) number of vessels responding to NE; *e*) number of vessels responding to NE by dilatation (light bars) or constriction (dark bars). Here and in Fig. 2: *1*) intact controls; *2*) injection of saline 2 h after thioglycolate; *3*) injection of PGP 2 h after thioglycolate; *4*) injection of PGP 15 min before thioglycolate. $p<0.001$ compared to: *intact group, +animals injected with saline 2 h after thioglycolate.

During step II we studied PGP-induced modification of mast cell reactivity during the development of inflammatory reaction. The number of experimental and control groups of animals and the time of peptide and thioglycolate injections were the same as in the previous series of experiments. The percent of degranulated mast cells with consideration for the degranulation degree was evaluated on film preparations of the mesentery under a light microscope.

The significance of differences was evaluated using Student's *t* test.

RESULTS

The reaction of lymph vessels to NE 2 h after inflammation induction (Fig. 1) corresponded to previous data [4] and was characterized by sharp prolongation of response latency, reduction in the number of contractions per minute, duration of the response and number of vessels responding to NE, and changed vascular tone: the response of lymph vessels under conditions of inflammation started by dilatation, but not by constriction (as in intact animals). The peptide injected 2 h after or 15 min before thioglycolate significantly corrected the mesenteric microcirculatory system: the parameters characterizing lymph vessel response to NE did not return to normal, but differed significantly from those under conditions of inflammation. The latency decreased significantly in the experimental groups; the number of vessels responding to NE application increased and the vascular tone changed (the overwhelming majority of vessels responded to NE by constriction). Hence, the peptide injected before and after thioglycolate significantly reduced the mesenteric capillary dysfunction in rats with experimental peritonitis.

Mast cells play an important role in the maintenance of tissue homeostasis, particularly under conditions of stress reaction and inflammatory response [7,8]. The function of these cells is characterized by a drastic increase in the level of secretory activity. We previously showed that injection of glyprolines before stress exposure significantly attenuated the stressogenic disorders in the rat mesenteric microcirculation [2]. Stabilization of mast cells by preinjection of ketotifene prevented stress-induced disorders in contractile activity of mesenteric lymph vessels and reduced stressogenic activation of mast cells [1]. Increased secretory activity of mast cells during inflammation suggested that the protective effect of PGP is mediated by inhibition of secretory activity of mast cells during inflammation-induced disorders of contractile activity of lymph vessels.

The number of degranulated cells in the mesentery increased 1.8 times ($p<0.001$; Fig. 2) 2 h after the development of peritonitis in experimental series II, the percentage of cells with slight degranulation decreased, while the percentage of cells with moderate and severe degranulation increased.

The peptide injected 15 min before induction of peritonitis reduced the number of degranulated cells virtually to normal level, degranulation was minor in the majority of cells.

On the other hand, injection of the peptide 2 h after thioglycolate did not decrease the number of degranulated cells and did not change cell distribution by the degree of degranulation.

Hence, PGP exhibited preventive and therapeutic effects on disordered contractile activity of rat mesenteric lymph vessels. The preventive effect of PGP was largely due to prevention of mast cell activation. Injection of PGP after thioglycolate did not inhibit

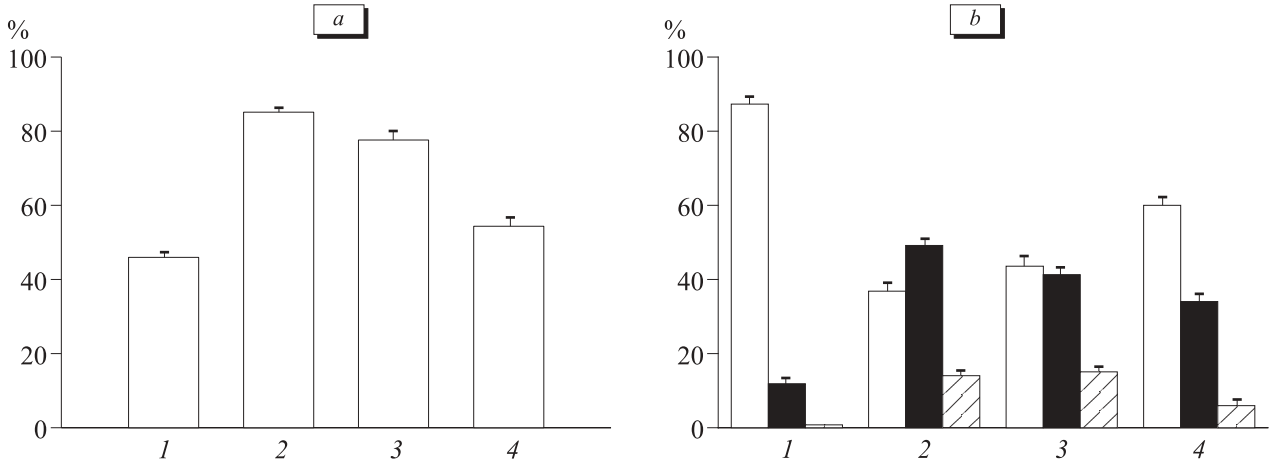

Fig. 2. Percent of degranulated cells (*a*) in rat mesentery and their distribution by the degree of degranulation (*b*). Light bars: mild degranulation; dark bars: moderate degranulation; cross-hatched bars: severe degranulation.

the secretory activity of mast cells, which suggests other mechanisms of the therapeutic effect of the peptide.

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