The Role of Protective Effects of Proline-Containing Peptides (PGP, PG, and GP) in Contractile Dysfunction of Mesenteric Lymphatic Vessels in Rats with Experimental Acute Peritonitis

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The development of acute peritonitis in rats induced by intraperitoneal injection of thioglycollate was accompanied by a decrease in contractile function of mesenteric lymphatic vessels and impaired response to norepinephrine. Administration of proline-containing peptides after induction of inflammation significantly decreased the severity of these disorders. Our results attest to the possibility of using peptides for the correction of mesenteric microcirculatory disturbances during inflammation.

Key Words: *inflammation; lymphatic vessels; glyprolines*

The lymphatic system plays an important role in the maintenance of tissue homeostasis, particularly under conditions of inflammation. The release of proinflammatory mediators is followed by dilation of vessels and increase in vascular permeability, which results in edema. Rhythmic contraction of lymphatic vessels is one of the major mechanisms underlying functional activity of the lymphatic system [7]. Mediators play a key role in modulation of function of lymphatic vessels during inflammation [6,10].

The family of short peptides containing glycine and proline (glyprolines) is characterized by a wide range of physiological activity. They maintain homeostasis in the gastric mucosa under ulcerogenic conditions, prevent microcirculatory disturbances [2], inhibit thrombus formation [8], and are involved in the regulation of vascular tone [1].

Our previous studies showed that stressogenic factors inhibit the contractile response of lymphatic vessels to norepinephrine (NE). Pretreatment with proline-containing peptides (PGP, PG, and GP) prevents the development of stress-induced microcirculatory disturbances [3,4].

Here we studied the effect of glyprolines on dysfunction of mesenteric lymphatic vessels in rats with acute peritonitis.

MATERIALS AND METHODS

Peritonitis in rats was induced by intraperitoneal injection of 2 ml 40% thioglycollate (Fluka) per 200 g body weight. Vital microscopy of mesenteric microcirculatory bed was performed at various stages of peritonitis (30 min and 2, 6, and 18 h after administration of thioglycollate) [3].

We studied the external characteristics of the mesentery, measured the volume of exudate in the abdominal cavity, and evaluated the response of lymphatic vessels to application of NE (10^{-6} M). The latency (time between application of NE and start of contractions), number of contractions, and length of contraction were recorded. The amplitude

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of contractions (percent of the initial diameter of vessels) was measured on a monitor. The percentage of NE-insensitive vessels was calculated.

Peptides (Institute of Molecular Genetics, Russian Academy of Sciences) in a dose of 3.7 μmol/kg were injected intraperitoneally 5 min after thioglycollate administration. Control animals received physiological saline or equivalent dose of glycine.

Experiments were performed on male outbred albino rats weighing 150-180 g. Our study complied with the requirements of the European Sci-

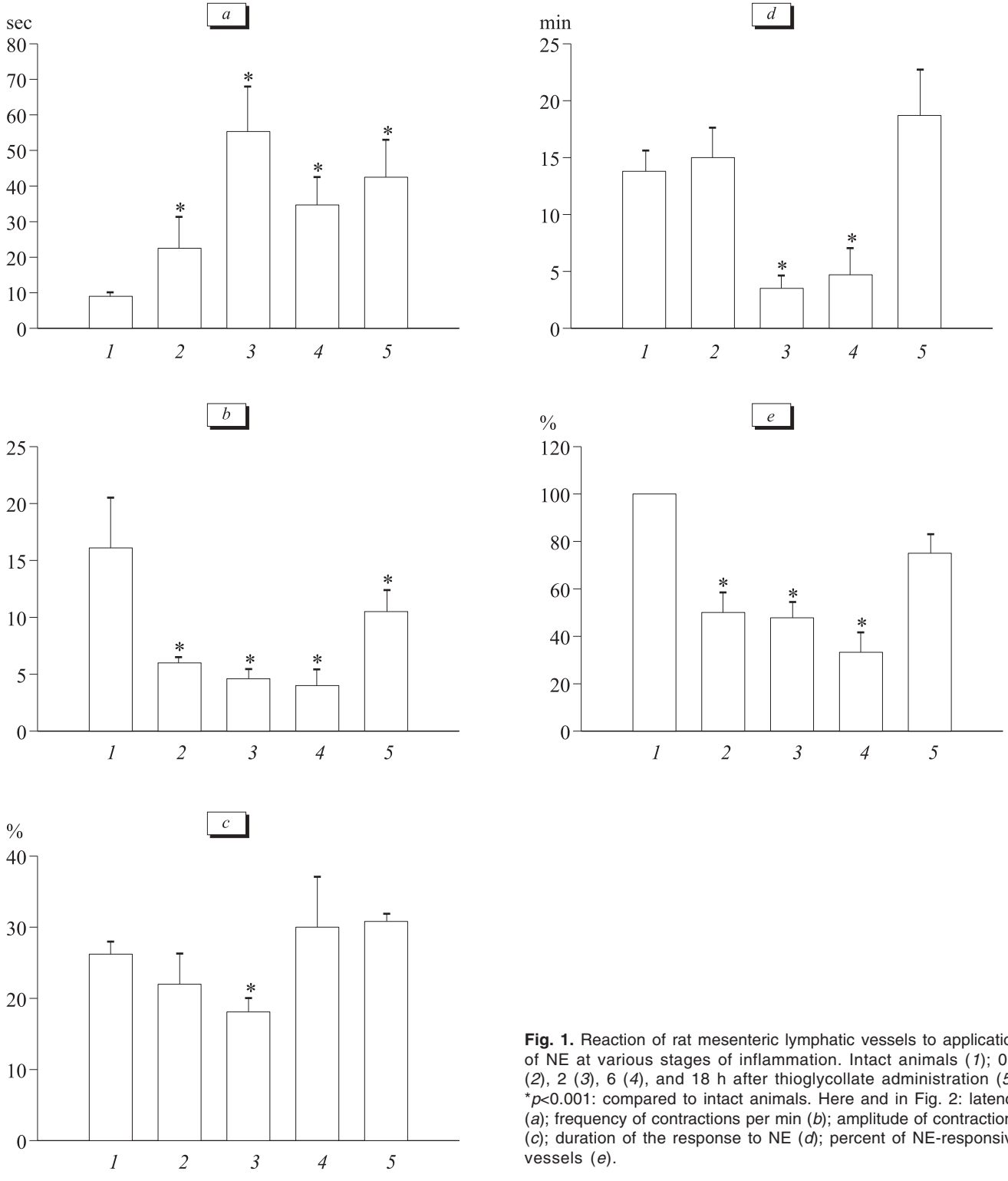

Fig. 1. Reaction of rat mesenteric lymphatic vessels to application of NE at various stages of inflammation. Intact animals (*1*); 0.5 (*2*), 2 (*3*), 6 (*4*), and 18 h after thioglycollate administration (*5*). **p*<0.001: compared to intact animals. Here and in Fig. 2: latency (*a*); frequency of contractions per min (*b*); amplitude of contractions (*c*); duration of the response to NE (*d*); percent of NE-responsive vessels (*e*).

entific Foundation ESF). The significance of differences was estimated by Student's *t* test.

RESULTS

Figure 1 illustrates the response of mesenteric lymphatic vessels to NE application 30 min and 2, 6, and 18 h after the induction of inflammation.

Treatment with NE was followed by short-term constriction and rhythmic contractions of lymphatic vessels in intact animals (latency 8.9±1.4 sec; 16.1±2.4 contractions per min; amplitude 26.2±18%; response duration 13.9±1.8 min).

The response of lymphatic vessels to NE significantly decreased after induction of inflammation. The ratio of responsive vessels sharply decreased (Fig. 1, *e*). Several lymphatic vessels were characterized by temporal suppression of the response to NE (from 30 min to 2 h). The response of vessels did not return to normal after increasing in the con-

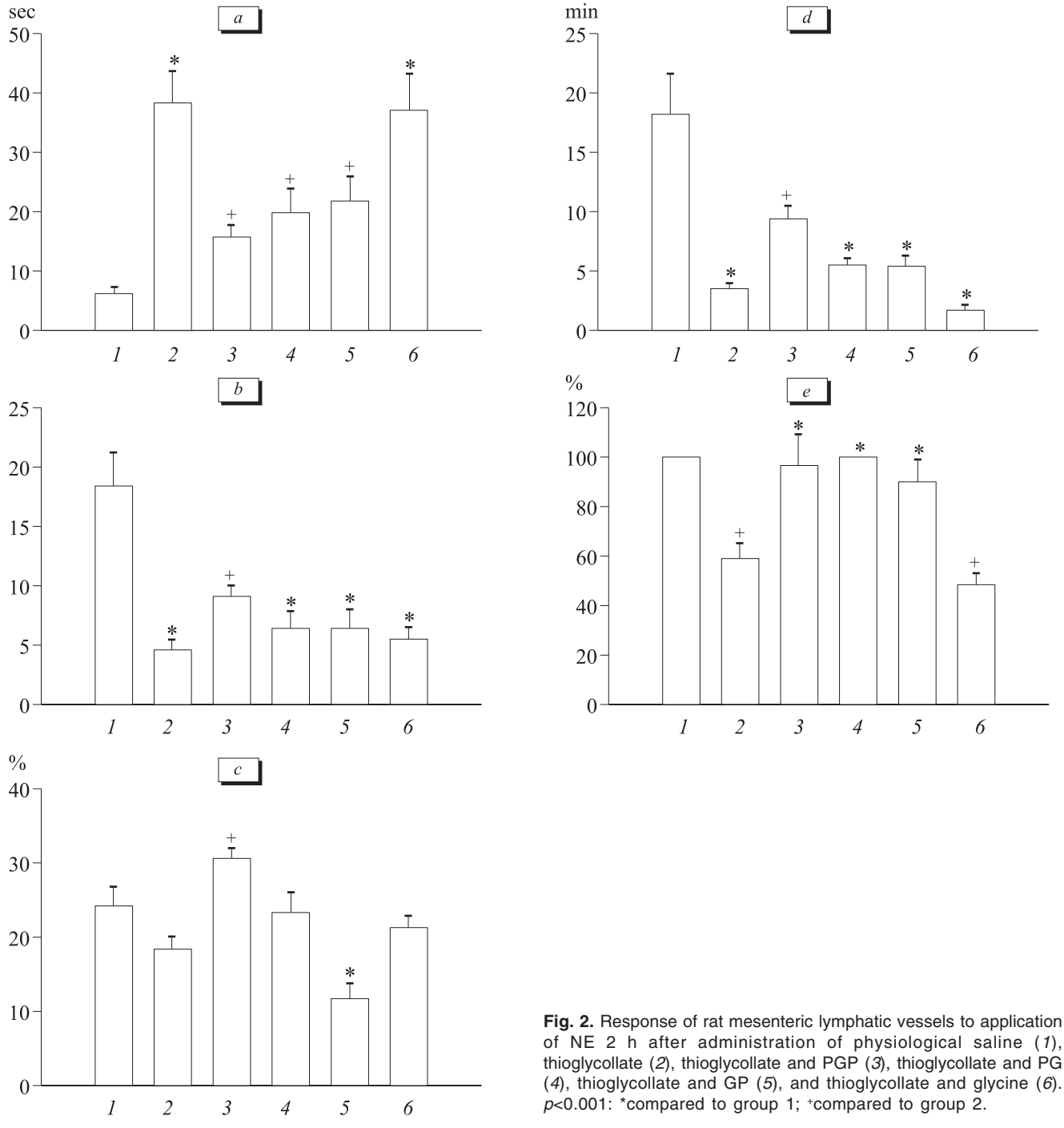

Fig. 2. Response of rat mesenteric lymphatic vessels to application of NE 2 h after administration of physiological saline (*1*), thioglycollate (*2*), thioglycollate and PGP (*3*), thioglycollate and PG (*4*), thioglycollate and GP (*5*), and thioglycollate and glycine (*6*). $p<0.001$: *compared to group 1; +compared to group 2.

centration of NE to 10^{-4} M. Moreover, the NE-induced response of lymphatic vessels in treated rats started from dilation. This reaction had a long latency. The observed dilation in many animals was not followed by contractions. It should be emphasized that control animals exhibited constriction of lymphatic vessels under these conditions. Contractile activity of NE-responsive vessels in treated rats significantly differed from that in control animals. We observed sharp lengthening of the response latency and decrease in its frequency and duration (Fig. 1, *a*, *b*, *e*). The amplitude of contractions little changed (Fig. 1, *c*).

The decrease in the response to NE was accompanied by stasis of lymphatic vessels and absence of spontaneously contracting vessels at various stages of inflammation. Intestinal tissues were pale or sometimes cyanotic. It was probably related to venous congestion. The induction of abdominal inflammation was accompanied by the appearance of considerable amounts of exudate (10-15 ml) with blood traces. Exudate was not found in control animals.

Therefore, induction of inflammation is followed by a decrease in contractile activity of lymphatic vessels. These changes were most pronounced 2 h after administration of an inflammation-inducing agent. The amplitude of lymphatic vessel contractions, duration of the response to NE, and number of responsive vessels in treated rats returned to normal after 18 h (Fig. 1, *c*, *d*, *e*). The latency of the response remained high, while the frequency of contractions was low. However, changes in the test parameters were less significant compared to those observed 2 h after induction of inflammation (Fig. 1, *a*, *b*).

In the next series, the animals received intraperitoneal injection of peptides (PGP, PG, and GP) 5 min after administration of the inflammation-inducing agent. The PGP metabolite glycine was given to rats of one group. The reaction of lymphatic vessels to application of NE was studied 2 h after induction of inflammation (Fig. 2).

Glyprolines had a protective effect on the mesenteric microcirculatory system and significantly decreased the severity of disturbances during inflammation. The volume of exudate in thioglycollate-treated animals decreased by 2 times after peptide treatment. PGP produced the most potent protective effect. Glyprolines had a strong corrective effect on vascular tone. After administration of peptides, a greater number of lymphatic vessels responded to NE by constriction, but not by dilation (injection of thioglycollate without peptide treatment). These peptides increased the number of NE-responsive vessels to the basal level (Fig. 2, *e*). Administration of glycine after thioglycollate injection had no correcting effect on contractile activity of mesenteric lymphatic vessels. The test parameters in treated rats practically did not differ from those in thioglycollate-treated animals (Fig. 2).

Our results show that administration of glyprolines immediately after treatment with inflammation inductor significantly improves function of the mesenteric microcirculatory bed in rats with experimental peritonitis. The mechanism underlying the protective effect of glyprolines on contractile activity of lymphatic vessels remains unclear. Our previous studies showed that the ability of glyprolines to decrease the severity of stressogenic microcirculatory disorders in rat mesentery is associated with the stabilizing effect on mast cells [5]. Mediators of these cells modulate vascular permeability and contribute to the development of edema during inflammation [9]. The decrease in secretory activity of mast cells under the influence of peptides probably improves contractile activity of lymphatic vessels on this model of inflammation.

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