Effects of Prolyl-Glycyl-Proline (PGP) Peptide on Disorders in Rat Mesenteric Lymphatic Vessel Function Induced by Injection of Substance 48/80

B. A. Umarova, N. S. Bondarenko, T. V. Lelekova, G. N. Kopylova, and G. E. Samonina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 10, pp. 428-431, October, 2011
Original article submitted April 21, 2010

Injection of substance 48/80 to rats led to dysfunction of mesenteric lymphatic microvessels, in particular inhibition of their contractility and modification of their reaction to norepinephrine. Injection of PGP peptide before and after substance 48/80 alleviated these disorders. The results indicated the possibility of peptide correction of lymphatic vessel dysfunction.

Key Words: *glyprolines; lymphatic vessels; substance 48/80*

The lymphatic systems is actively involved in the maintenance of homeostasis, including the tissue homeostasis. Due to rhythmic contractions, the lymphatic microvessels (LV) work as the drainage system under conditions of homeostasis disorders, this preventing accumulation of tissue fluid and formation of edemas [9]. We have previously shown that stress and inflammatory stimuli caused disorders in the LV contractile activity, consisting in inhibition of their reaction to norepinephrine application. Regulatory peptides of the glyproline family reduce these disorders or prevent their development [5].

Disorders in LV function in stress and inflammation are to a certain measure associated with mast cell stimulation and release of vasoactive mediators from them [7].

We have shown that intraperitoneal injection of substance 48/80 (nonselective stimulator of mast cells) also led to disorders of this kind. Stabilization of mast cells with ketotiphene completely prevented the stressogenic disorders in LV function [4]. We suggested that mast cell stabilization could be a mechanism of realization of PGP peptide protective effects towards the mesenteric microvessels. On the other hand, *in vitro* experiments showed that glyprolines stabilized mast cells preventing their stimulation with $ACTH_{1-24}$ fragment (sinactene), but not with substance 48/80 [3]. Hence, manifestation of the glyproline protective effects under conditions of mast cells stimulation with substance 48/80 suggested the existence of other mechanisms of the glyproline effects [6].

We studied the effects of PGP tripeptide (a glyproline family representative) on disorders in the rat mesenteric LV reactivity induced by stimulation of mast cells with substance 48/80.

MATERIALS AND METHODS

The study was carried out in acute experiments on male outbred albino rats (n=30; 180-220 g) narcotized with urethane (2.25 g/kg).

After laparotomy, the animal was placed on a warmed (37°C) table; a mobilized intestinal loop with the mesentery was placed on the microscope table. The image of the examined mesenteric fragment was visualized on the Matrix device monitor. The following parameters of LV response to norepinephrine (10^{-6} M) application were evaluated: the latent period (from the moment of norepinephrine application to the beginning of rhythmic contractions); number and amplitude

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

0007-4888/12/1524-0447

of contractions during the first minute of the response; length of response; number of spontaneously active vessels, frequency and amplitude of contractions. Vessels with the reaction starting by constriction or dilatation were recorded.

PGP peptide (synthesized at Institute of Molecular Genetics, the Russian Academy of Sciences) was injected intraperitoneally in a dose of 3.7 mmol/kg 15 min before or 15 min after injection of substance 48/80. Substance 48/80 (Sigma) was injected intramuscularly (1 mg/kg). Animals injected with saline served as the control.

All experiments on animals were carried out in accordance with ethic philosophy and regulations recommended by the European Scientific Foundation (ESF) and Declaration on Humane Attitude to Animals. The results were processed by nonparametric Mann–Whitney test using Statistica software.

RESULTS

Preventive effects of PGP were studied in experimental series 1. The animals were intraperitoneally injected with saline and after 15 min, with substance 48/80. The other group of animals received PGP 15 min before substance 48/80.

In order to evaluate the therapeutic effect of PGP, PGP (or saline) was injected to animals 15 min after substance 48/80, i. e. in the presence of unfolding microcirculatory disorders.

Controls were injected with saline twice at 15-min interval.

In controls, LV reacted by rhythmic contractions to norepinephrine application (Fig. 1). The latent period (LP) of the response was 12.4±1.7 sec, with 13.8±1.4 contractions during the first minute, the amplitude of contractions being 23.1±2.3% of the initial diameter of the vessel and response duration 6.1±0.9 min. The percentage of spontaneously contracting vessels was 36.2±4% of the total number of studied vessels, while 75.9±5.1% vessels responded to norepinephrine.

Preinjection with PGP (before substance 48/80) reduced the severity of virtually all disorders in LV function (Fig. 1). All parameters of their response to norepinephrine application in fact did not differ from the control. The number of spontaneously active LV increased by 77.6% in comparison with animals injected with saline and substance 48/80, the frequency of contractions of these vessels increased by 69.7% ($p<0.05$) and their amplitude increased by 2.5 times ($p<0.05$). The number of LV reacting to norepinephrine application increased by 27.6%. LP of LV response was shorter by 2.5 times ($p<0.05$), the frequency and amplitude of contractions were higher by 51 and 28% ($p<0.05$), and duration of response was longer by 19%. The number of LV reacting by constriction or dilatation also remained close to normal.

Injection of substance 48/80 15 min after saline reduced the number of LV responding to norepinephrine application and to changes in the parameters of their contractile activity (Fig. 1). The LP increased by 3.2 times ($p<0.05$), the frequency, amplitude, and duration of the vascular contractile response decreased by 49% ($p<0.01$), 35%, and 39% ($p<0.05$), respectively. In addition, the percentage of spontaneously contracting vessels decreased by 52.7%, while the frequency and amplitude of contractions dropped (by 2.7 times and 2-fold, respectively; $p<0.01$). The majority of vessels responded by dilatation, but not by constriction to epinephrine. All these changes indicated inhibition of LV function. Similar changes in LV contractility, caused by substance 48/80, were observed after its injection 15 min before saline (Fig. 1).

Injection of PGP after substance 48/80 also led to improvement of the mesenteric microcirculatory status (Fig. 1). The amplitude of contractions of spontaneously active LV normalized. The percentage of vessels responding to norepinephrine increased by 43.4% ($p<0.05$). The frequency of LV contractions increased by 28%, amplitude by 67% ($p<0.01$), and duration of the response by 16%. The number of vessels reacting by constriction or dilatation was close to normal, similarly as in the previous series.

Hence, PGP exhibited preventive and therapeutic effects on disorders of the rat mesenteric LV contractility, caused by mast cell stimulation with substance 48/80. It is a well-known fact that mast cell mediators modulate LV contractility [8,9].

As PGP does not protect mast cells from stimulation with substance 48/80 [3], it seems that the protective effect of the peptide under conditions of disorders induced by injection of substance 48/80 was due to not stabilization of mast cells, but to other regulatory properties. Realization of these properties at the tissue and organ levels seemed to prevent the disorders unfolding under the effects of mast cell mediators. The protective effect of PGP not mediated through mast cell stabilization could include peptide regulation of the vascular tone [1], endothelium permeability maintenance, maintenance of adequate bloodflow [2], and improvement of cell and tissue resistance to oxidative stress [5].

Detection of the therapeutic effect of PGP additionally confirms the existence of mechanisms of its effect not associated with mast cell stabilization, as the peptide was injected when mast cells had been stimulated.

The study was supported by the Russian Foundation for Basic Research (grant No. 09-04-00669-a).

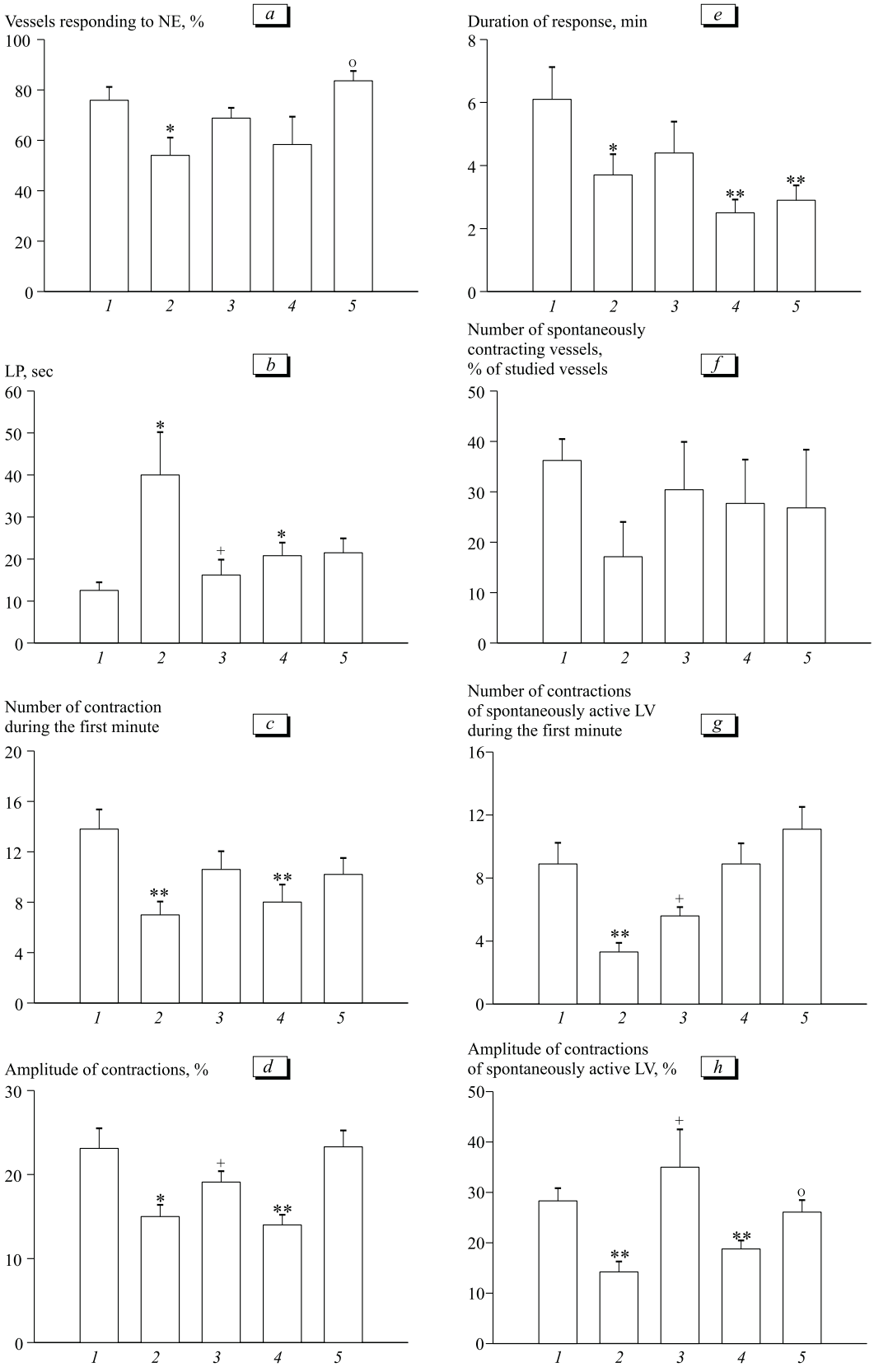

Fig. 1. Contractility parameters of the rat mesenteric LV responding to norepinephrine (NE; *a*-*e*) and spontaneously contracting mesenteric LV (*f*-*h*). *1*) control animals; *2*) saline+substance 48/80; *3*) PGP+substance 48/80; *4*) substance 48/80+saline; *5*) substance 48/80+PGP. **p*<0.05, ***p*<0.01 in comparison with group 1; ^{+}p<0.05 in comparison with group 2; ^{o}p<0.05, ^{oo}p<0.01 in comparison with group 4.

REFERENCES

1. Z. V. Bakaeva, K. E. Badmaeva, I. Yu. Sergeev, and G. E. Samonina, *Byull. Eksp. Biol. Med.*, **135**, No. 4, 390-393 (2003).
2. S. E. Zhuikova and G. E. Samonina, *Uspekhi Fiziol. Nauk*, **33**, No. 1, 77-87 (2002).
3. G. N. Kopylova, E. A. Smirnova, L. Ts. Sanzhieva, *et al.*, *Byull. Eksp. Biol. Med.*, **136**, No. 11, 497-499 (2003).
4. G. N. Kopylova, E. A. Smirnova, L. Ts. Sanzhieva, *et al.*, *Vestn. Moskovsk. Gos. Univers.*, Ser. 16. *Biol*., No. 2, 6-9 (2006).
5. E. R. Safarova, S. I. Shramm, Yu. A. Zolotaryov, and N. F. Myasoedov, *Byull. Eksp. Biol. Med.*, **135**, No. 3, 309-313 (2003).
6. B. A. Umarova, G. N. Kopylova, T. V. Lelekova, *et al.*, *Neirokhim.*, **25**, Nos. 1-2, 119-123 (2008).
7. B. A. Umarova, G. N. Kopylova, E. A. Smirnova, *et al.*, *Byull. Eksp. Biol. Med.*, **136**, No. 10, 371-373 (2003).
8. J. L. Fox and P. Y. von der Weid, *Br. J. Pharmacol.*, **136**, No. 8, 1210-1218 (2002).
9. P. Y. von der Weid, *Aliment. Pharmacol. Ther.*, **15**, No. 8, 1115-1129 (2001).
10. T. F. Wu, W. K. MacNaughton, and P. Y. von der Weid, *Mem. Inst. Oswaldo Cruz*, **100**, Suppl. 1, 107-110 (2005).