PHYSIOLOGY

Effect of Acute and Moderate Repeated Stress on Disturbances in Reactivity of Mesenteric Lymphatic Vessels during Inflammation in Rats

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We studied the effect of acute (single immobilization for 1 h) and repeated (daily immobilization for 1 min, 5 days) moderate stress on disturbances in contractility of mesenteric lymphatic vessels in rats with experimental peritonitis. Acute stress was shown to potentiate, while moderate repeated stress attenuate the effect of inflammatory stimulus. It can be hypothesized that moderate repeated stress improves adaptive capacities of the organism, which manifests in reduction or prevention of dysfunction in contractile activity of lymphatic vessels.

Key Words: *inflammation; stress; lymphatic vessels*

Chronic and severe stress can induce serious disturbances in various systems of the organism, which results in the development of diseases. The pathogenesis of these diseases is often associated with the inflammatory process. Strong evidence exists that stress exposure increases susceptibility of the organism to inflammatory agents. These changes are related to the exhaustion of stress-liming systems and reduced release of anti-inflammatory mediators [4,5]. However, moderate and short-term stress can activate the endogenous protective mechanisms improving organism's resistance to subsequent exposure to adverse factors [6]. For example, repeated restraint stress has a strong inhibitory effect on vascular endothelial permeability (major component of the inflammatory response) [9]. Pre-exposure to moderate stress prevents gastric ulceration in rats under conditions of severe stress [10].

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Lymphatic vessels have close functional relations with the hematopoietic, nervous, and other systems in the organism and are involved in the adaptive response to stress and inflammation. Rhythmic activity of lymphatic vessels plays a crucial role in the negative and positive course of the diseases (*e.g.*, inflammation) [8,11].

Our previous studies showed that the development of inflammation in rats (experimental peritonitis) is followed by paradoxical changes in the tone of mesenteric lymphatic vessels in response to standard contraction-inducing agent norepinephrine [3]. The majority of vessels responded by dilation without subsequent contraction (instead of expected constriction and phasic contractions). Changes in the tone of lymphatic vessels were accompanied by impairment of vascular contractility (increase in the latency, decrease in the frequency of contractions, and shortening of the response). Similar changes were observed in rats after single exposure to various stress factors [1]. However, the effect of moderate repeated stress on the function of lymphatic vessels remains unknown.

Stress is an etiological or risk factor for the development of inflammation. The effects of acute and moderate stress are mediated by various physiological mechanisms. Here we studied the influence of stress factors on reactivity of mesenteric lymphatic vessels in rats with experimental peritonitis.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats (n=70) weighing 150-180 g. The study was conducted in accordance with the recommendations of the European Science Foundation (ESF).

Inflammation (acute peritonitis) in rats was induced by an intraperitoneal injection of 40% sodium thioglycollate (1 ml per 100 g body weight; Fluka) [7].

The mesenteric microcirculatory bed was visually examined by intravital microscopy. Contractility of mesenteric lymphatic vessels was evaluated from the response to norepinephrine [2]. Under normal conditions, norepinephrine (10^{-6} M) causes constriction of lymphatic vessels and increases the frequency of contractions. Abnormal response to norepinephrine attests to dysfunction of lymphatic vessel. We evaluated the latency of response (period between application of norepinephrine and start of contractions), frequency of contractions over the 1st minute, and duration of the response. Changes in the vascular tone and number of norepinephrine-responding vessels were recorded. The mesentery was examined visually.

The following two types of stress exposure were used: single acute stress (1-h immobilization in the supine position on a table) and moderate repeated stress (daily immobilization for 1 min, 5 days). After stress, the animals received an intraperitoneal injection of thioglycollate. Reactivity of lymphatic vessels was evaluated 2 h after the induction of inflammation. The animals exposed to moderate repeated stress were treated with thioglycollate immediately or 1 day after the last episode of immobilization.

The significance of differences was estimated by Student's t test.

RESULTS

In series I, contractile activity of mesenteric lymphatic vessels in rats was studied after acute and moderate repeated stress (Fig. 1). Thioglycollate (inflammatory agent) caused dysfunction of lymphatic vessels, which manifested in an increase in the latency of contractions, decrease in the frequency of contractions (1st minute of the response), and shortening of contractions

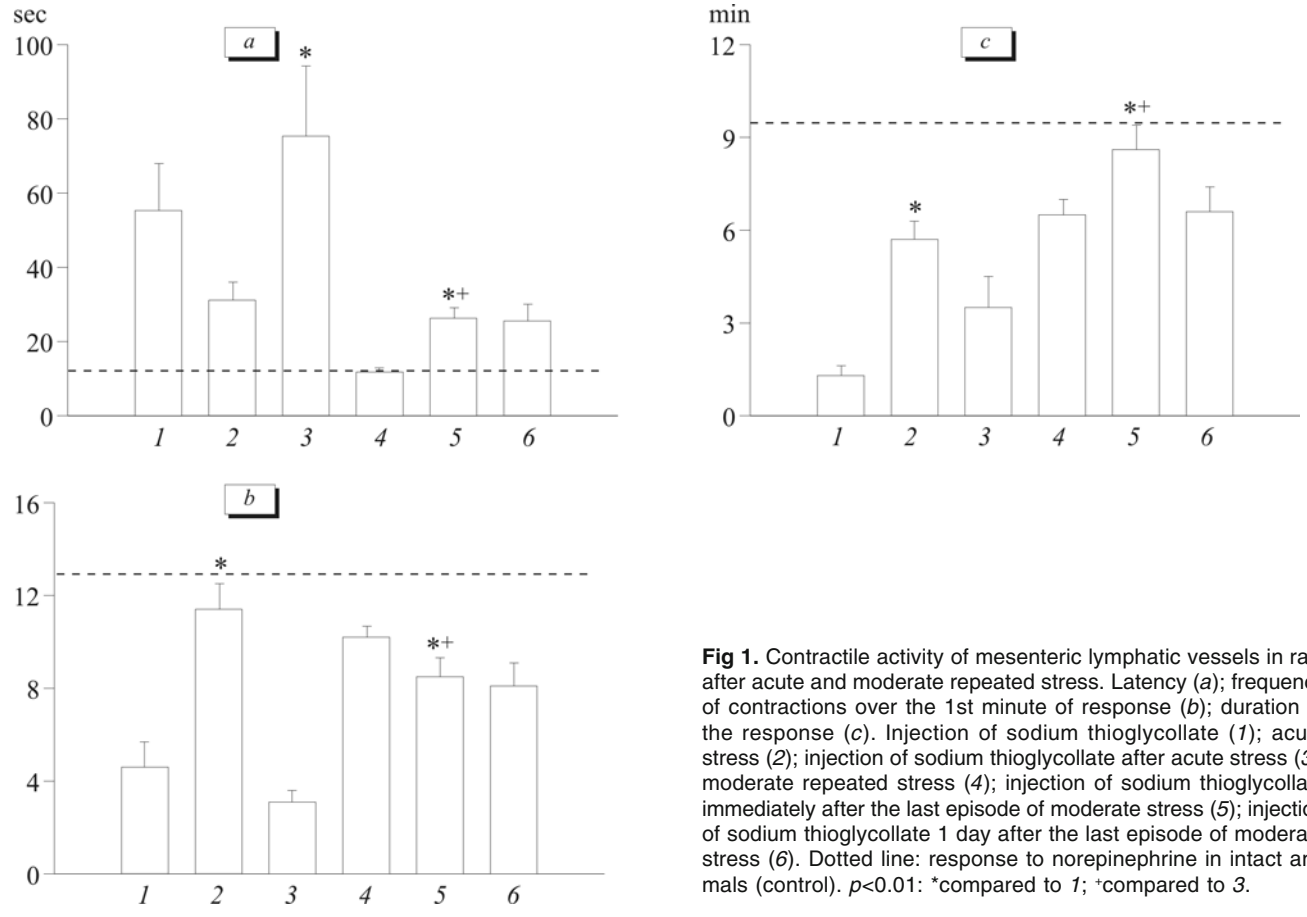

Fig 1. Contractile activity of mesenteric lymphatic vessels in rats after acute and moderate repeated stress. Latency (*a*); frequency of contractions over the 1st minute of response (*b*); duration of the response (*c*). Injection of sodium thioglycollate (*1*); acute stress (*2*); injection of sodium thioglycollate after acute stress (*3*); moderate repeated stress (*4*); injection of sodium thioglycollate immediately after the last episode of moderate stress (*5*); injection of sodium thioglycollate 1 day after the last episode of moderate stress (*6*). Dotted line: response to norepinephrine in intact animals (control). p<0.01: *compared to *1*; +compared to *3*.

(as compared to the control). The exposure to acute and moderate stress was followed by similar, but less significant changes in lymphatic microcirculation (Fig. 1). Contractile activity of lymphatic vessels in rats after acute or moderate repeated stress was shown to differ significantly from that in intact animals.

Inflammation was accompanied by a significant decrease in the number of spontaneously contracting vessels. By contrast, the number of vessels not responding to norepinephrine was elevated under these conditions (Fig. 2). The number of spontaneously contracting vessels in stressed rats practically did not differ from that in intact animals. We revealed only a slight increase in the number of vessels not responding to norepinephrine (Fig. 2).

The inflammatory agent had a greater effect on the organism compared to stress. The tone of lymphatic vessels did not change in stressed animals. The majority of vessels exhibited a strong response to norepinephrine under conditions of inflammation. In stressed animals, vasoconstriction was the primary response of most vessels to norepinephrine (Fig. 3).

In series II, we studied the effect of acute and repeated stress on microcirculatory disturbances under conditions of inflammation. Acute stress aggravated microvascular dysfunction in the mesenteric lymphatic bed under conditions of inflammation. The vascular response to norepinephrine was characterized by a greater increase in the latency, decrease in the frequency of vascular contractions, and short time of the reaction (as compared to animals with inflammation; Fig. 1). The induction of inflammation in animals of the acute stress group was accompanied by a sharp decrease in the number of spontaneously contracting vessels and increase in the number of vessels not responding to norepinephrine (Fig. 2). The tone of vessels was modified under these conditions. We revealed an increase in the number of vessels exhibiting a dilatory response to norepinephrine (Fig. 3).

Moderate repeated stress had another effect on contractile activity of lymphatic vessels in animals with inflammation. The response latency decreased, while the frequency and duration of contractions were elevated in animals of this group (as compared to rats with inflammation; Fig. 1).

It should be emphasized that administration of thioglycollate after moderate repeated stress was not followed by a decrease in the number of spontaneously contracting vessels and increase in the amount of norepinephrine-insensitive vessels (as differentiated from inflammation; Fig. 2). However, the initial reaction of vessels to norepinephrine was dilation (Fig. 3). This probably results from induction of mechanisms mediating contractions and tone of lymphatic vessels during stress and inflammation.

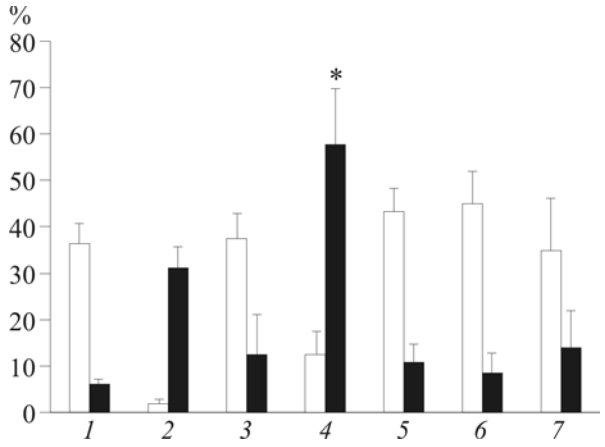

Fig. 2. Number of spontaneously contracting (light bars) and norepinephrine-insensitive (dark bars) mesenteric lymphatic vessels in rats after acute and moderate repeated stress. Here and in Fig. 3: intact rats (*1*); injection of sodium thioglycollate (*2*); acute stress (*3*); injection of sodium thioglycollate after acute stress (*4*); moderate repeated stress (*5*); injection of sodium thioglycollate immediately after the last episode of moderate stress (*6*); injection of sodium thioglycollate 1 day after the last episode of moderate stress (*7*). **p*<0.05 compared to spontaneously contracting vessels during acute stress.

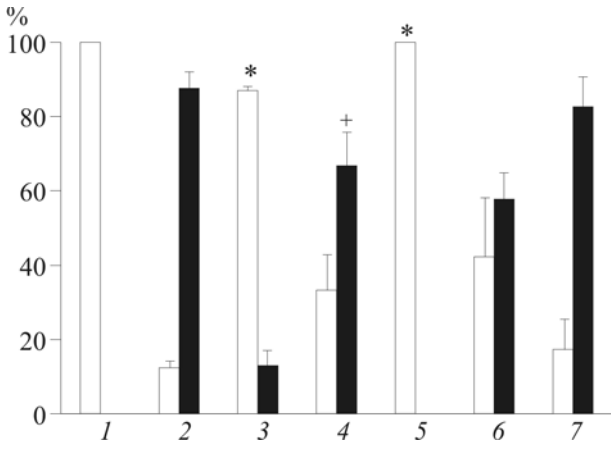

Fig. 3. Tone of mesenteric lymphatic vessels in rats after acute and moderate repeated stress. Light bars, constriction; dark bars, dilation. **p*<0.001 compared to injection of sodium thioglycollate; +*p*<0.001 compared to acute stress.

Vascular contractility did not differ in animals receiving thioglycollate immediately or 1 day after moderate stress (Fig. 1). Hence, the effect of moderate repeated stress persists for at least 24 h.

We conclude that functional activity of mesenteric lymphatic vessels depends not only on the strength of adverse factors, but also on the adaptive capacities of the organism. Severe acute stress was shown to potentiate, while moderate repeated stress attenuate the effect of inflammatory stimulus. Moderate repeated stress probably improves the adaptive capacities of the organism. It is manifested in the reduction or prevention of dysfunction in contractile activity of lymphatic vessels. Moderate repeated stress can be considered as

a factor increasing resistance of lymphatic vessels to subsequent exposure to the inflammatory stimulus.

REFERENCES

1. G. N. Kopylova, E. A. Smirnova, L. Ts. Sanzhieva, *et al*., *Byull. Eksp. Biol. Med.*, **136**, No. 11, 497-499 (2003).
2. I. Yu. Sergeev and T. V. Lelekova, *Vestn. Mos. Gos. Univer. Ser. Biol*., No. 4, 18-21 (2000).
3. B. A. Umarova, T. V. Lelekova, G. N. Kopylova, *et al*., *Byull. Eksp. Biol. Med.*, **142**, No. 9, 248-251 (2006).
4. P. H. Black, *Brain Behav. Immun*., **16**, No. 6, 622-653 (2002).
5. S. M. Collins, *Am. J. Physiol. Gastrointest. Liver Physiol*., **280**, No. 3, G315-G318 (2001).
6. C. Kiank, M. Entleutner, B. Fürll, *et al*., *Shock*, **27**, No. 3, 305-311 (2007).
7. G. Pejler, *Inflamm. Res*., **48**, No. 6, 344-350 (1999).
8. M. S. Pepper and M. Skobe, *J. Cell Biol*., **163**, No. 2, 209-213 (2003).
9. H. J. Strausbaugh, P. G. Green, M. F. Dallman, and J. D. Levine, *Eur. J. Neurosci*., **17**, No. 4, 805-812 (2003).
10. A. Tanaka, R. Hatazawa, Y. Takahira, *et al*., *Dig. Dis. Sci*., **52**, No. 2, 478-487 (2007).
11. T. F. Wu, W. K. MacNaughton, and P. Y. von der Weid, *Mem. Inst. Oswaldo Cruz*, **100**, Suppl. 1, 107-110 (2005).