Mechanisms Underlying the Effect of *E. coli* Endotoxin on Contractile Function of Lymphatic Vessels

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E. coli endotoxin decreased the amplitude and frequency of spontaneous phasic contractions in isolated bovine mesenteric lymphatic vessels. This substance in a concentration of 5 mg/liter blocked spontaneous contractions and reduced tonic tension of smooth muscle cells. The dilatory effect of endotoxin on lymphatic vessels was primarily realized via stimulation of synthesis of NO and prostacyclin by endotheliocytes.

Key Words: *lymphatic vessels; smooth muscle cells; endotoxin; vasodilation; prostacyclin*

The major function of lymphatic vessels is drainage of excess extracellular fluid and proteins to the blood system [3]. Lymph transport is mainly provided by phasic contractions of segments in lymphatic vessels (lymphangions) and, to a lesser extent, by extralymphatic forces depending on tonic tension of myocytes [1,15]. Various bioactive substances produced in tissues under physiological and pathological conditions modulate the state of smooth muscle cells (SMC) in lymphatic vessels (tonic tension, frequency and amplitude of phasic contractions).

In adult humans the number of bacteria in the large intestine reaches 10^{12} bacteria/liter. The total weight of intestinal bacteria is 1.5-2.0 kg [10]. Various substances are released from bacterial cells into the intestine during natural death of bacteria. Several substances, including membrane fragments of gram-negative bacteria (endotoxins or lipopolysaccharides, LPS), penetrate the intestinal mucosa and appear in lymphatic capillaries. It should be emphasized that gaps between endothelial cells in lymphatic capillaries is tens and hundreds times greater than in blood vessels. The rate of endotoxin release into lymph flow markedly increases during inflammation and ischemia of the intestinal wall [2,4].

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Endotoxins affect smooth muscles in blood vessels. Recent studies evaluated the mechanisms underlying the influence of endotoxins on various blood vessels. However, the effect of endotoxins on lymphatic vessels is poorly understood. Changes in lymph flow were studied after *in vivo* administration of LPS to experimental animals [7,8]. The response of lymphatic microvessels to *in vitro* treatment with LPS was also investigated [14]. The mechanism underlying the effect of endotoxins on large lymphatic vessels is little studied. It is clear that disturbances in transport function of lymphatic vessels accompanying the course of pathological processes (including those produced by bacteria) play an important role and often determine their outcome. Here we studied the mechanism underlying the direct effect of *E. coli* endotoxin on transport function of bovine mesenteric lymphatic vessels.

MATERIALS AND METHODS

Experiments were performed on isolated bovine mesenteric lymphatic vessels with a diameter of 2-3 mm (47 segments of lymphatic vessels from 17 healthy animals). The use of isolated vessels allowed us to exclude the effects of nervous and systemic humoral factors on contractile activity of myocytes. The reaction of these cells to endotoxin was evaluated. Lymphatic vessels were isolated 10-15 min after exsanguination and stored in physiological saline at 2-4°C. The

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study was conducted 4-6 h after sampling. Rings (3-4 mm, n=64) were excised from the middle part of the muscular cuff of the lymphangion [1]. Changes in tension were recorded in the natural circular direction. Some preparations were denuded by air pumping through the vessel for 2 min (n=12). The preparations were placed in an experimental chamber. Krebs physiological saline containing (in mmol/liter) 120.4 NaCl, 5.9 KCl, 1.2 NaH_2PO_4, 15.5 $NaHCO_3$, 2.5 $CaCl_2$, 1.2 $MgCl_2$, and 5.5 glucose saturated with gas mixture (95% O_2+5% CO_2) at a constant temperature (37°C) was continuously delivered through the chamber at a flow rate of 1 ml/min. Contractile activity of preparations was studied using 6MKh1S mechanoelectrical transducers. The results were recorded on an H-3031/3 automatic recorder.

When spontaneous activity of preparations in physiological saline became stable, segments of lymphatic vessels were treated with *E. coli* LPS in concentrations of 1, 5, and 10 mg/liter. LPS was dissolved in DMSO (ISN) and diluted with Krebs solution to the required concentrations. The role of the endothelium in LPS-induced reactions of SMC was estimated by deendothelialization of preparations. Vasodilating substances formed under the influence of endotoxin were identified using L-NAME (Nω-nitro-L-arginine methyl ester, 5×10^{-5} mol/liter, ICN) and indomethacin (2×10^{-5} mol/liter, Sigma) blocking the synthesis of nitric oxide and prostacyclin, respectively. Precontraction of preparations was evoked by norepinephrine in a concentration of 1×10^{-6} mol/liter (ICN). The results were analyzed by Student's t test.

RESULTS

Tonic tension of segments in lymphatic vessels usually becomes stable 8-10 min after the start of measurements. Spontaneous contractile activity was recorded 10-15 min later. The strength and frequency of phasic contractions were 7.40±0.76 mN and 6.30±0.61 min^{-1}, respectively. The strength of phasic contractions decreased on the 1st minute after introduction of LPS into the solution (1 mg/liter, n=22). Spontaneous contractions disappeared 5-6 min after treatment. Tonic tension of preparations progressively decreased in the follow-up period (by 7.30±0.88% compared to the baseline level, Fig. 1, *a*). Stimulation of lymphatic vessels with reduced tension by a single electrical impulse (field stimulation) evoked phasic contraction, whose amplitude was lower compared to the initial level. Endotoxin in a concentration of 5 mg/liter rapidly decreased the strength and frequency of phasic contractions in the vascular segments (n=8). Spontaneous activity was terminated by the 2nd minute after treatment with LPS. Tonic tension of preparations rapidly decreased (19.20±1.63% of the baseline level). LPS in a concentration of 10 mg/liter produced a more potent inhibitory effect on SMC of lymphatic vessels (n=17). Electrostimulation did not evoke phasic contractions after treatment with LPS (Fig. 1, *b*).

Endothelial cells modulate the response of vascular smooth muscles to a variety of bioactive substances. The next series was performed on deendothelialized preparations (n=12). The effect of LPS on these preparations was less pronounced. Phasic contractions of deendothelialized preparations did not disappear over 10 min after administration of LPS in a concentration of 1 mg/liter. However, endotoxin in a concentration of 10 mg/liter blocked phasic contractions (5-6 min after treatment). Myocyte tension in deendothelialized preparations decreased less significantly compared to intact segments (14.30±2.55% of the baseline level).

L-NAME was introduced into the solution with intact segments to evaluate the role of nitric oxide (NO) in dilation of lymphatic vessels under the influence of endotoxin. Under these conditions LPS-induced vasodilation was less pronounced compared to intact preparations. After treatment with the NO synthase blocker, endotoxin in a concentration of 1 mg/liter slightly decreased the frequency and amplitude of phasic contractions (14.60±3.34% of the baseline level) in deendothelialized lymphatic vessels. Spontaneous phasic contractions were not terminated.

In the next series we studied the tonic response of SMC in lymphatic vessels to LPS (n=19, Fig. 2). In-

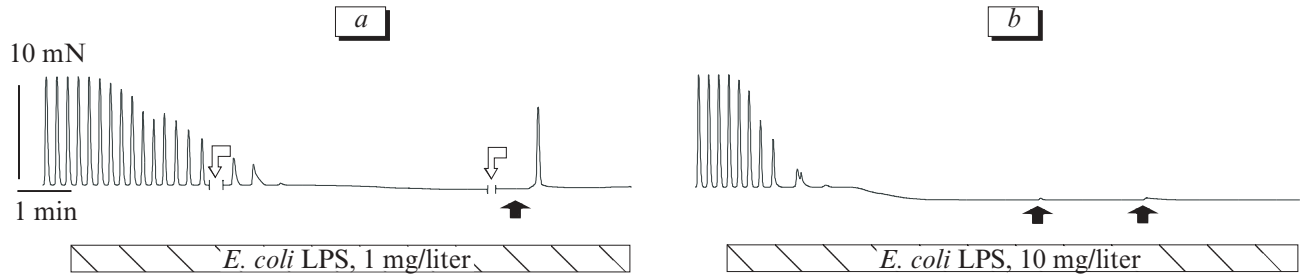

Fig 1. Two recordings of the effect produced by *E. coli* LPS in concentrations of 1 (*a*) and 10 mg/liter (*b*) on contractile activity of lymphatic vessels. Breakpoints (upper arrows): stop of recording (5 min). Lower arrows: single electrical impulses (20 V, 50 msec).

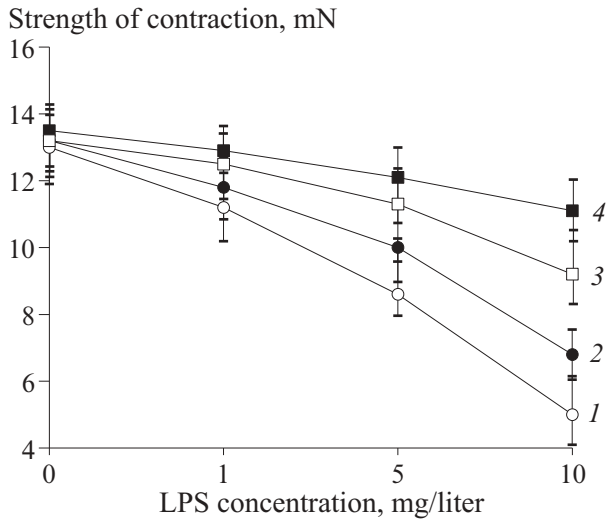

Fig. 2. Dilation of norepinephrine-precontracted intact and deendothelialized lymphatic vessels under the influence of *E. coli* endotoxin (Krebs solution) and after pretreatment with L-NAME (NO synthase blocker) and indomethacin (prostacyclin synthesis blocker): intact lymphatic vessel (*1*), intact vessel and L-NAME (*2*), deendothelialized vessel (*3*), intact vessel and indomethacin (*4*).

tact and deendothelialized segments of lymphatic vessels were pretreated with norepinephrine. Both preparations were characterized by termination of phasic contractile activity and appearance of stable tonic contractions. LPS in concentrations of 1, 5, and 10 mg/liter was introduced into the solution. In a special series of experiments on intact lymphatic vessels, L-NAME or indomethacin was introduced in combination with norepinephrine. LPS produced a strong relaxing effect on SMC of lymphatic vessels (Fig. 2). The amplitude of relaxation induced by LPS in various concentrations was maximum in intact segments of lymphatic vessels. The dilatory effect of LPS was less pronounced after pretreatment with L-NAME. Administration of indomethacin attenuated the dilatory effect of endotoxin. The degree of relaxation was minimum in deendothelialized segments of lymphatic vessels.

Our results indicate that *E. coli* endotoxin produces the dilatory effect on phasic and tonic contractions of myocytes in lymphatic vessels. Published data suggest that endotoxins stimulate the formation of cytokines in various tissues. However, cytokine synthesis takes long time [5,6,10]. In our experiments the dilatory response developed rapidly, which indicates that endotoxin produces a direct effect on lymphatic vessels. The amplitude of LPS-induced relaxation in deendothelialized segments of lymphatic vessels was much lower than in intact preparations. Therefore, the reaction of SMC to endotoxin is modulated by endotheliocytes (similarly to blood vessels) [13].

The endothelium of lymphatic vessels can secrete various bioactive substances, including NO and prostacyclin. Application of NO synthase blocker (L-NAME) to lymphatic vessels with intact endothelium markedly decreased the amplitude of LPS-induced relaxation. Administration of prostacyclin synthesis blocker indomethacin abolished the relaxing effect of endotoxins on lymphatic vessels.

These findings show that *E. coli* endotoxin produces a potent dilatory effect on SMC of lymphatic vessels, which is mediated by direct stimulation of synthesis of NO and prostacyclin in endotheliocytes.

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