DOI 10.1007/s10517-017-3939-z
Bulletin of Experimental Biology and Medicine, Vol. 164, No. 2, December, 2017 PHYSIOLOGY

Inhibitory Effect of Interferons on Contractive Activity of Bovine Mesenteric Lymphatic Vessels and Nodes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 164, No. 8, pp. 145-149, August, 2017
Original article submitted March 23, 2017

We studied the effect of IFNα-2b and IFNβ-1a on phasic and tonic contractions of isolated bovine mesenteric lymphatic vessels and nodes. IFNα-2b and IFNβ-1a in concentrations of 250-1000 U/ml produced dose-dependent negative chronotropic and inotropic effects on spontaneous phasic contractions and tonus of lymphatic vessels and nodes. In de-endothelialized lymphatic vessels and nodes, IFNα-2b and IFNβ-1a in the same concentrations had less pronounced inhibitory effect on spontaneous contraction and tonus. L-NAME (100 μM) and charybdotoxin (0.1 μM with 0.5 μM apamine) significantly attenuated the inhibitory effect of IFNα-2b on phasic and tonic contractions of lymph nodes. L-NAME (100 μM) and indomethacin (10 μM) significantly reduced the IFNα-2b-induced inhibitory effect on phasic and tonic contractions of lymph node. These results indicate that IFNα-2b and IFNβ-1a have a pronounced inhibitory effect on the phasic and tonic contractions of bovine mesenteric lymphatic vessels and nodes. The responses are endothelium-dependent and are determined by production of NO and endothelium-dependent hyperpolarizing factor by endotheliocytes in lymphatic vessels and by production of NO and prostacyclin by endotheliocytes in the lymphatic nodes.

Key Words: *lymphatic vessels; lymphatic nodes; smooth muscle cells; endotheliocytes; NO*

Interferon therapy in the form of monotherapy or in combination with other drugs is a modern trend in the treatment of viral infections and some malignant tumors [6,12]. IFN is a group of low-molecular proteins and glycoproteins released by cells in response to infection with viruses and other microorganisms. They are the main factors of nonspecific resistance and have different protective effects [3]. Human IFN are divided into several groups, among them IFNα-2b and IFNβ-1a are of particular importance. Both IFN belong to type I and bind to cell surface receptor known as IFNα receptor (IFNAR), which leads to the formation of an active signal complex triggering phosphorylation and activation of various protein kinases and formation of various bioactive substances, *e.g.* protein kinase C and phosphoinositol. Receptors to type 1 IFN were found practically in all cell types, including smooth muscle and endothelial cells [11].

As IFN have protein nature, they cannot be use orally and are administered subcutaneously or intramuscularly. Large IFN molecules (molecular weight ≥20 kDa) administered parenterally cannot enter directly into blood vessels, but like other high-molecular compounds are absorbed into lymphatic capillaries and then transported through the lymphatic vessels (LV) and lymph nodes (LN) in contact with endothelial and smooth muscle cells. It is generally recognized that contractile activity of smooth muscle cells in the LV wall and LN capsule is a critical factor of lymph flow [1,2,13].

We studied the effect of IFNα-2b and IFNβ-1a on active transport function of LV and LN.

MATERIALS AND METHODS

Bovine mesenteric LV with a diameter of 1.5-2.0 mm (n=24) and LV (n=21) were used in the experiments.

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Strips of LN capsule (length 15 mm, width 3 mm, thickness 500-600 μ; *n*=36) oriented perpendicular to the long axis of the node and LV rings (width 2-2.5 mm; *n*=33) were prepared. The experiments were performed in a thermostated chamber constantly perfused with physiological saline (in mM: 120.4 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 15.5 $NaHCO_3$, and 11.5 glucose) saturated with a gas mixture consisting of 95% O_2 and 5% CO_2 (pH 7.35-7.40) at 37±0.2°C. LV rings and LN capsule strips were preliminary stretched to create tension corresponding to transmural pressure of 5 cm H_2O. After 30-min adaptation, contractile activity of the preparations was recorded using strain gauge FORT-10 (WPI) operated in the isometric mode as the force transducer. The data was recorded on the computer during the experiment with an analog-to-digital converter (MD-155) and the Labmaster software [6].

We used solutions containing 250 and 500 U/ml Intron A (IFNα-2b; Merck Sharp & Dohme Corp.), 600 U/ml Rebif (IFNβ-1a; Merck Serono S.p.A.), 100 μM L-NAME (ICN Biomedicals), 10 μM indomethacin (ICN Biomedicals), 0.1 μM charibdotoxin (Sigma-Aldrich), and 0.5 μM apamin (Sigma-Aldrich). Indomethacin was previously dissolved in equimolar Na_2CO_3; other drugs were dissolved in bidistilled water.

The results were statistically processed using Statistica 6.1.478 software. As the data had normal distribution, they were presented as mean values and standard deviation (*M*±*SD*). Significance of differences was evaluated using Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

After 30-min incubation in the thermostatic chamber with physiological saline, stable tonus and spontaneous phasic contractions were observed in 29 LV rings and 32 LN capsule strips. All tests were carried out only on preparations with spontaneous phasic activity. IFNα-2b in a concentration of 250 U/ml produced an inhibitory effect on contractile activity of smooth muscle cells of LV and LN. After 3-4-min exposure to IFNα-2b, a decrease in the tonus of LV was recorded, the tonus of LN decreased in 6-8 min. Against the background of reduced tonus, the amplitude and frequency of spontaneous phasic contractions also decreased. Spontaneous phasic contractile activity of LV and LN ceased in 11.3±1.6 and 18.60±2.47 min, respectively. Removal of IFNα-2b from the bathing solution led to slow recovery of contractile activity of LV and LN.

Increasing the concentration of IFNα-2b in the solution to 500 U/ml (theoretically calculated concentration of IFNα-2b in the extracellular fluid of a human weighing 70 kg after administration of the therapeutic dose without considering pharmacodynamics) led to a rapid development of negative inotropic and chronotropic effects. A decrease in tonus and amplitude of phasic contractions of LV and LN was observed during the first minutes of exposure. Spontaneous phasic contractile activity against the background of reduced tonus in LV ceased on 4.30±0.55 min and in LN on 7.80±1.03 min. By this time, the tonus of LV and LN decreased by 24.30±3.28 and 18.80±2.46%, respectively, from the initial value. The inhibitory effect of IFNα-2b in a dose of 500 U/ml on LV and LN was reversible, but recovery of parameters of phasic contractile activity and tonus was slow. IFNα-2b in a concentration of 1000 U/ml produced potent inhibitory effect within the first minutes: the tonus rapidly decreased and spontaneous phasic activity stopped in 2-3 min.

The reactions of LV and LN to IFNβ-1a were similar to the effects of IFNα-2b. IFNβ-1a in a concentration of 600 U/ml (50% of theoretically calculated concentration in the extracellular fluid of a human

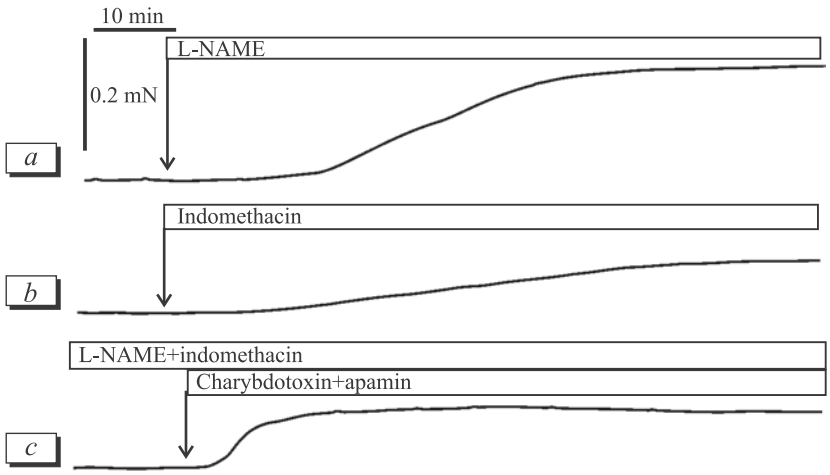

Fig. 1. Changes in the tonus of bovine mesenteric LV caused by different inhibitors of vasodilator production against the background of IFNα-2b (500 U/ml) application. *a*) Response to NO synthase blocker L-NAME; *b*) response to indomethacin; *c*) response to apamin and charybdotoxin against the background of L-NAME and indomethacin application.

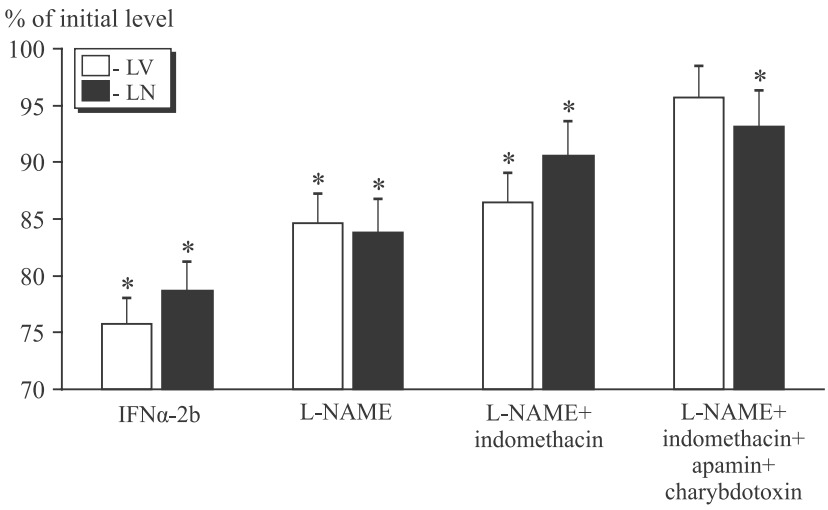

Fig. 2. Change in the tonus of bovine mesenteric LV and LN under the action of IFNα-2b (500 U/ml) and the administration of the test substances against the background of IFNα-2b application. *p<0.05 in comparison with the initial value (100%).

weighing 70 kg after administration of the therapeutic dose without consideration of preparation pharmacodynamics) reduced the tonus of LV and LN preparations ass soon as on the first minutes after application; the amplitude and frequency of spontaneous phasic contractions also decreased. Spontaneous phasic contractile activity against the background of reduced tonus in LV and LN ceased in 4.80±0.62 and 6.90±1.14 min, respectively. By this time, the tonus of LV decreased by 19.60±2.74%, and LN by 23.70±3.13% from the initial level. The inhibitory effect of IFNβ-1a in a concentration of 1200 U/ml on LV and LN was reversible, but the aftereffect was strongly pronounced: in 1 h after removal of IFNβ-1a from the solution, the tonus of the preparations was not restored and phasic contractions of LV and LN were irregular, rare, and had low amplitude.

In de-endothelialized LV and LN, IFNα-2b (500 U/ml) and IFNβ-1a (600 U/ml) produced weak inhibitory effect: the decrease in the tonus was less pronounced in comparison with intact preparations; spontaneous phasic activity did not cease, though the rhythm and amplitude of contractions somewhat decreased. These data suggested that the inhibitory effect of both IFN on contractile activity of smooth muscle cells in LV and LN is largely endothelium-dependent. Further experiments were aimed at deciphering of the mechanisms underlying the action of IFN on LV and LN. Since the effects of both IFN on the contractile function of smooth muscle cells in LV and LN were similar, the study with IFNα-2b (500 U/ml) and IFNβ-1a (600 U/ml) was performed by the same protocol. We proceeded from the classical notion that relaxation of smooth muscles of vessels can be induced by NO, prostacyclin and endothelial hyperpolarizing factor produced by the endothelium [8,10,12] and applied inhibitors and blockers of these three signaling pathways sequentially (or simultaneously).

After complete cessation of spontaneous phasic activity under the influence of IFN, NO synthase inhibitor L-NAME was added to the solution. After application of L-NAME, the tonus of LV and LN increased slowly and after 8-10 min was set at a new, relatively stable level (Fig. 1, *a*). Application of L-NAME against the background of IFN significantly increased the tonus of LV preparation by 40.50±4.51% from the value of dilatation caused by IFNα-2b. In LN, the effect of L-NAME was less pronounced: 22.70±3.17% from the value of dilatation caused by the use of IFNα-2b (p<0.05).

Possible participation of prostacyclin in the mechanism of the realization of the inhibitory effect of IFN on LV and LN was studied in two variants: 1) additions of cyclooxygenase inhibitor indomethacin to the solution after relaxation of the preparation under influence of IFN; 2) simultaneous application of L-NAME and indomethacin; and then the responses to L-NAME and to two substances were compared. The analysis of the results showed that in the LN, application of indomethacin against the background of IFN and L-NAME led to a pronounced additional increase in tonus (by 31.90±3.86%; p<0.05; Fig. 1, *b*). In LV, administration of indomethacin in similar experiments was accompanied by a slight increase in tonus (by 7.80±1.31%).

At the final stage, possible participation of endothelial hyperpolarizing factor in the realization of the inhibitory effect of IFN on smooth muscle cells of LV and LN was determined. According to published reports, this role can be played by arachidonic acid metabolite epoxyeicosatrienoic acid [4,7]. The effects of endothelial hyperpolarizing factor are difficult to analyze and a typical approach in such studies is the use of blockers of Ca^{2+} sensitive K^+ channels involved in the relaxation of smooth muscles under the action of endothelial hyperpolarizing factor [10]. In our experi-

ments, production of NO and prostacyclin induced by IFN was preliminarily blocked by NO synthase blocker L-NAME and cyclooxygenase inhibitor indomethacin. Under these conditions, IFN-induced relaxation could be sustained only by production of endothelial hyperpolarizing factor. After inhibition NO and prostacyclin synthesis and stabilization of the tonus of the samples at a new level, apamin (blocker of low-conductivity Ca^{2+} sensitive K^+ channels) and charybdotoxin (blocker of medium- and high-conductivity Ca^{2+} sensitive K^+ channels) were applied [5,10]. Administration of these substances was followed by a rapid and pronounced increase in the tonus of the preparations. This effect was strongly expressed in LV (38.00±4.22% of the amplitude of relaxation caused by the use of IFN) and far lesser potent in LN (10.30±1.28%, $p<0.05$; Figs. 1 and 2). Previous studies have demonstrated that endothelial hyperpolarizing factor leads to relaxation of vascular smooth muscle via activation of these two types of Ca^{2+} sensitive K^+ channels [2,4,9]. In our study, their inhibition led to a decrease in the amplitude of relaxation of LV and LN, which suggests that IFN stimulates production of endothelial hyperpolarizing factor by endothelial cells in LV and LN.

Our findings suggest that the inhibitory effect of IFNα-2b and IFNβ-1a on LV and LN is endothelium-dependent. Both IFN in the interaction with LV and LN activate in the endothelial cells three signaling pathways that lead to relaxation of smooth muscle cells. In the LV, the NO-dependent dilatation mechanism and the mechanism triggered by the endothelial hyperpolarizing factor predominate. In LN IFNs stimulate the production of mainly NO and prostacyclin by endotheliocytes. The role of the endothelial hyperpolarizing factor in IFN-induced dilatation of smooth muscle cells of LN is minimal.

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