Heparin Inhibits Contraction of Smooth Muscle Cells in Lymphatic Vessels

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 1, pp. 7-10, January, 2010
Original article submitted July 8, 2009

Heparin in concentrations of 10-50 U/ml produced a decrease in the amplitude and frequency of phasic contractions of smooth muscle cells of bovine mesenteric lymphatic vessels. Vascular wall tension was reduced under these conditions. The endothelium-dependent effect of heparin is realized via an increase in the production of nitric oxide by endothelial cells and, to a lesser degree, via the stimulation of prostacyclin synthesis.

Key Words: *lymphatic vessels; smooth muscle cells; heparin; endothelium; nitric oxide*

Heparin, one of the major endogenous substances with potent anticoagulant activity, is extensively used in medical practice. Heparin is synthesized by mast cells of the connective tissue. These cells are located in various organs of animals and humans, including the lungs, liver, and intestinal mucosa. A considerable number of mast cells are found within and around the walls of lymphatic vessels (LV) [1]. As a natural anticoagulant, heparin is used for the prevention and therapy of thromboses. Heparin in a single dose up to 400 U/kg is recommended in myocardial infarction 12]. Anticoagulant activity of heparin is associated with activation of antithrombin. A monomolecular layer of thrombin covers the endothelial surface directed toward the vascular lumen. Therefore, heparin increases the negative charge of this surface. It contributes to the decrease in platelet adhesion and aggregation (*i.e.*, prevention of thrombus formation). Heparin concentration on the surface of vascular endothelial cells is 100-fold higher than in blood plasma [7].

Published data indicate that heparin may cause vasodilation. Previous studies revealed that the development of hypotension after intravenous infusion of heparin is related to massive release of histamine into the blood flow [4]. Chronic administration of heparin in medical purposes has the hypotensive effect, which is mainly associated with an increase in nitric oxide (NO) concentration in blood plasma [3]. At the same time, some investigators did not reveal significant changes in NO concentration after heparin infusion [9].

The methods of endolymphatic and lymphotropic therapy are widely used in medical practice. A concept of active lymph transport was proposed. Hence, studying the effect of heparin on LV is of considerable importance. It is interesting to evaluate the influence of heparin on contractile activity of smooth muscle cells (SMC) in the LV wall, which determines the transport function of these structures.

Here we evaluated the effect of heparin on SMC of isolated LV. This approach allowed us to exclude the *in vivo* indirect reactions. The mechanism for action of heparin on LV SMC was studied in details.

MATERIALS AND METHODS

Experiments were performed on circular segments of isolated LV from bovine mesentery (n=43). The diameter of LV was 2-3 mm. Circular segments (width 2-2.5 mm) were prepared from the central part of lymphangion muscular cuff. The study was conducted in a working chamber under continuous flow conditions (1.5 ml/min). The modified physiological Krebs solution consisted of 120.4 mmol/liter NaCl,

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0007-4888/10/1491-0004

5.9 mmol/liter KCl, 2.5 mmol/liter $CaCl_2$, 1.2 mmol/liter $MgCl_2$, 1.2 mmol/liter NaH_2PO_4, 15.5 mmol/liter $NaHCO_3$, and 11.5 mmol/liter glucose. Physiological saline was continuously saturated with carbogen (95% O_2 and 5% CO_2) and used for oxygenation and pH maintenance (7.35-7.40). The initial tension of the lymphatic vessel wall was adjusted in accordance with the Laplace's law (correspondence to a transmural pressure of 6 cm H_2O) [2]. Temperature of the washing solution was maintained at 37°C. Contractile activity of LV SMC was evaluated 30 min after the start of the experiments using a FORT-10 tension sensor (WPI). The signal was amplified, delivered from a sensor to ADC, and recorded with Labmaster software. Heparin solutions (specified concentrations) and test solutions were prepared *ex tempore*. The required amount of study substance was dissolved in Krebs solution. The following test substances were used: sodium heparin (10-50 U/ml, Gedeon Richter), N^{ω}-nitro-L-arginine methyl ester (100 μmol/liter, ICN Biomedicals), methylene blue (10 μmol/liter, Sigma), glybenclamide (1 μmol/liter, ICN Biomedicals), and indomethacin (10 μmol/liter, ICN Biomedicals).

The range of heparin concentrations (10-50 U/ml) was selected from possible content of heparin in blood plasma and lymph, which may increase due to degranulation of mast cells or therapeutic treatment with exogenous heparin. We took into account that heparin in a dose of 400 U/kg is injected intravenously for the prevention of thrombosis in patients. Under these conditions, the concentration of heparin in blood plasma reaches 10 U/ml [6,11].

The results were analyzed by methods of variation statistics. The significance of differences between the frequency and amplitude of phasic contractions and tonic tension of vascular segments was evaluated by nonparametric Mann–Whitney *U* test. The differences were significant at $p \leq 0.05$.

RESULTS

Spontaneous phasic contractions were observed in all segments of LV (n=43). The mean amplitude and frequency of contractions of LV segments were 4.90±0.41 mN and 5.20±0.55 min^{-1}, respectively. Addition of heparin (10 U/ml) to bathing solution was followed by a decrease in the amplitude and frequency of phasic contractions in intact segments of LV (n=14). Tonic tension of LV segments was reduced after treatment with heparin in this concentration (Fig. 1).

Heparin in concentrations of 25 (n=8) and 50 U/ml (n=11) had a stronger inhibitory effect on SMC of LV. The amplitude and frequency of spontaneous phasic contractions were significantly reduced under these conditions (Fig. 2). Addition of heparin in a concentration of 50 U/ml by the 15th-20th minute of treatment led to termination of spontaneous phasic contractions and significant decrease in the tension of vascular segments.

In vivo experiments on rats showed that 88% exogenous heparin are bound to endothelium of blood vessels (4 min after intravenous infusion of this compound) [7]. The endothelium of LV does not differ from the endothelium of blood vessels by anticoagulant activity and by the capacity to adsorb heparin [8]. NO synthase blocker L-NAME was used to confirm or to exclude the involvement of endothelial NO in the dilatory response of LV SMC to heparin. L-NAME added to physiological saline slightly increased the frequency of spontaneous phasic contractions of LV segments (n=22), which confirmed basal production of NO by endothelial cells of LV. NO synthase blocker had a strong modulatory effect on the reaction of LV SMC to heparin in concentrations of 10, 25, and 50 U/ml. The inhibitory effect of heparin was revealed during the later period. Moreover, changes in the amplitude and frequency of phasic contractions of SMC

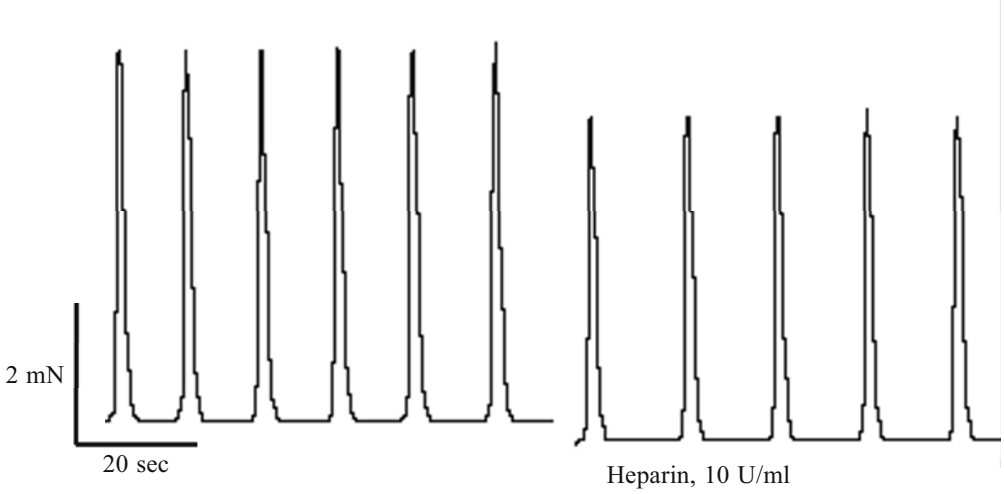

Fig. 1. Contractile activity of LV SMC under the influence of heparin (10 U/ml). Basal activity (*1*); 5 min after addition of heparin (*2*).

were less pronounced under these conditions (as compared to physiological saline; Fig. 3).

We studied the mechanism for action of NO from endothelial cells of LV on SMC. Methylene blue (blocker of soluble guanylate cyclase in SMC) was added to the bathing saline. Heparin in a concentration of 25 U/ml was administered to the solution 15 min after addition of methylene blue. Heparin inhibited contractile activity of LV SMC. However, the effect of heparin under specified conditions was much less pronounced compared to that in physiological saline (Fig. 3).

Since cAMP produced during stimulation of soluble guanylate cyclase affects SMC via activation of membrane ATP-sensitive K^+ channels on SMC membrane, glybenclamide was added to the solution to evaluate the mechanism of heparin-produced changes [5]. After addition of glybenclamide, the inhibitory effect of heparin on contractile activity of LV SMC was much lower compared to the influence of heparin in physiological saline on myocyte contractility (Fig. 3).

Under certain conditions, the endothelium can produce prostacyclin, which causes vasodilation. To confirm or to exclude this mechanism of heparin action on LV, a cyclooxygenase blocker indomethacin was added to the solution 10 min before addition of heparin [10]. The inhibitory effect of heparin was less pronounced after addition of indomethacin (significant differences in the amplitude and frequency; Fig. 3).

Thus, heparin has a strong vasodilatory effect on LV. Heparin decreases the amplitude and frequency of spontaneous phasic contractions. Tonic tension of LV SMC also decreased under these conditions. The inhibitory effect of heparin on LV SMC is mainly realized via the endothelium-dependent mechanism. Heparin activates constitutive NO synthase. NO increases cGMP production due to activation of soluble guanylate cyclase. cGMP induces opening of membrane ATP-sensitive K^+ channels in SMC. These changes are followed by hyperpolarization and relaxation of SMC. The dilatory effect of heparin is partly mediated by activation of cyclooxygenase and synthesis of prostaglandin.

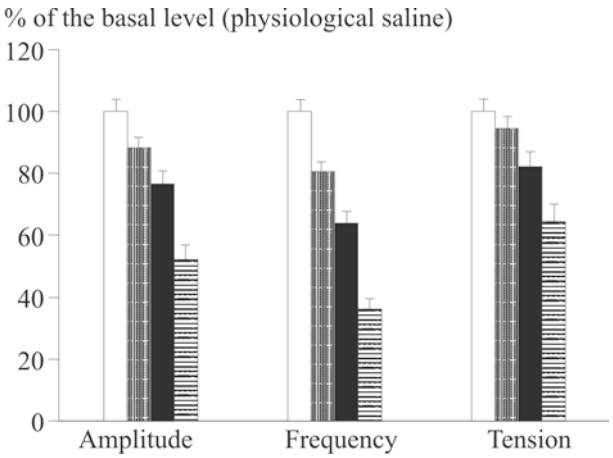

Fig. 2. Contractile activity of LV SMC in physiological saline (light bars) and 5 min after addition of heparin in concentrations of 10 (vertical shading), 25 (dark bars), and 50 U/ml (horizontal shading).

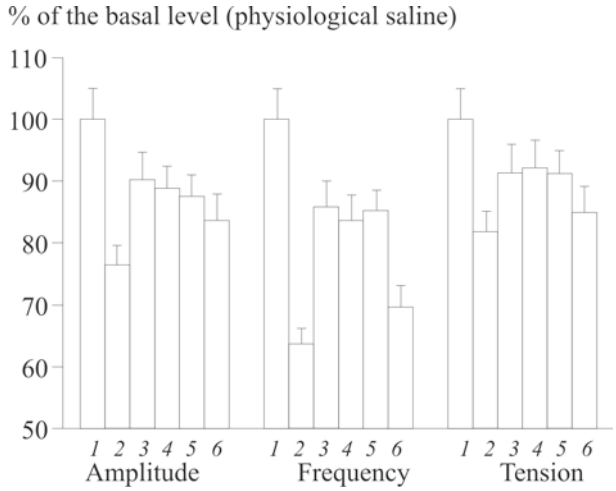

Fig. 3. Contractile activity of LV SMC in physiological saline (light bars) and 5 min after addition of heparin (25 U/ml) in the case of pretreatment with L-NAME, methylene blue, glybenclamide, and indomethacin. Physiological saline (*1*); heparin (*2*); L-NAME+heparin (*3*); methylene blue+heparin (*4*); glybenclamide+heparin (*5*); indomethacin+heparin (*6*).

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