Effect of Histamine on Contractile Activity of Smooth Muscles in Bovine Mesenteric Lymph Nodes

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The effects of histamine and mechanisms of its action on the capsular smooth muscle cells of mesenteric lymph nodes were examined on isolated capsular strips under isometric conditions. Histamine (1×10^{-8}-5×10^{-7} M) decreased the tone of capsular smooth muscle cells and the frequency of phasic contractions. At high concentrations (more than 5×10^{-6} M), histamine increased the amplitude and frequency of phasic contractions against the background of increased tonic stress. The effects of histamine were dose-dependent and were realized via direct stimulation of H_1- and H_2-receptors on the membrane of smooth muscle cells.

Key Words: *lymph nodes; smooth muscle cells; histamine; H_1- and H_2-receptors*

The lymph produced in organs and tissues travels across a single, but most frequently through several lymph nodes (LN) [1,6,13]. Apart from proteins and electrolytes, the lymph contains various substances including those with high biological activity. Their number and concentration increase dramatically during inflammation and allergic reactions accompanied by degranulation of the mast cells available in large amount in the walls of lymphatic vessels and in LN [6]. Histamine is the first agent to appear in tissues involved in inflammatory and allergic reactions [10]. In addition to antigen-dependent degranulation of the mast cells, there is a large moiety of physical and chemical histamine liberators, which induce degranulation of the mast cells via the non-immunological mechanisms [10].

The key role of histamine in the control of lymph transport is widely accepted; it is manifested not only in modulation of the contractile activity of smooth muscles in the lymphatic vessels [14], but also in the changes of lymph flow and composition during its pumping across LN [11,15]. Moreover, the effects and the mechanisms of action of histamine on contractile function of capsular smooth muscle cells (CSMC) in LN, the unique "crossroads" on the pathways of the antigens and immunocompetent cells [5,12,13], are far from being clear. However, the contractile activity of CSMC can pronouncedly change the hydrodynamic resistance of LN and its active pumping function which greatly determine the rate of development and intensity of the immune response [9,12].

This work was designed to study the effects and mechanisms of the directly applied histamine in CSMC.

MATERIALS AND METHODS

The experiments have been carried out on the strips cut of the capsules isolated from mesenteric bovine LN (n=56). The strips were cut perpendicularly to LN long axis. The length and width of the strips were 15 and 4 mm, respectively. In the bath chamber, the strips were continuously perfused with physiological solution containing (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 oxygenated with 95% O_2 and 5% CO_2 (pH 7.35-7.40). The temperature of the preparation was stabilized with a BT-5-1 Thermostat (Termex) at the level of 37.0±0.2°C. Initial tonic stress of the capsular strips were applied according to the level of intranodal pressure of 3 cm H_2O calculated with Laplace

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formula for a spherical node yielding [8]. Recording of CSMC contractions was started 45 min after the onset of experiment with a FORT-10 strain sensor (WPI) under isometric conditions. The data were continuously fed to PC with an MD-155 digitizer. The test solutions with histamine (10^{-9}-10^{-4} M), diphenhydramine (10^{-6} M), cimetidine (10^{-6} M), 2-pyridylethylamine (10^{-8}-10^{-5} M), and dimaprit (10^{-8}-10^{-5} M) were prepared immediately before the experiments by dissolving these substances (all reagents were from Sigma-Aldrich) in the above physiological solution.

Since the responses of blood and lymphatic vessels to histamine are rather complicated due to the presence of several subtypes of histamine receptors on the membranes of smooth muscle and endothelial cells, and due to ability of stimulated endothelium to produce and release NO and prostaglandins [4], we mechanically removed and eliminated the cortical substance and endothelial cells of the subcapsular sinus from LN capsule. Removal and elimination of the endothelial (littoral) cells of LN subcapsular sinus were evidenced by addition of acetylcholine (10^{-5} M, Sigma-Aldrich) and then sodium nitroprusside (10^{-5} M, Sigma-Aldrich) to physiological solution. The absence of CMSC response to acetylcholine and the positive response to sodium nitroprusside indicated adequacy of de-endothelization [7].

Contraction frequency, amplitude of phasic contractions, and tonic stress of LN smooth muscle strips were processed statistically using non-parametric Mann–Whitney U test at $p \leq 0.05$.

RESULTS

Under control conditions, the strips of LN capsule demonstrated spontaneous contractile activity [2]. In more than 80% preparations, these contractions were regular. In this study, we assessed responsiveness of the strips characterized by rhythmic phasic contractile activity maintained at the background of stable tonic stress (n=47). The amplitude and frequency of phasic contractions were 1.5-2.1 mN (1.8±0.2 mN) and 0.8-1.2 min^{-1} (1.0±0.1 min^{-1}), respectively.

The minimal concentration of histamine (10^{-8} M) changing the parameters of CSMC contractile activity was determined. Within the concentration range of 1×10^{-8} to 5×10^{-7} M, histamine reduced the amplitude of spontaneous phasic contractions and slightly diminished the tonic stress of the strips (in 40% cases the tonic stress changed insignificantly). When applied in concentration of 10^{-7} M, histamine decreased muscular tone as well as the amplitude and frequency of phasic contractions (Fig. 1, *a*). These effects slowly increased with time: on 5 min postapplication, the negative inotropic effect of histamine was 13.4±2.5%, while on minute 10 it increased to 18.3±2.4% compared to initial tonic stress. Higher concentrations of histamine (10^{-6}-10^{-5}M) produced a biphasic effect: during the first 3-4 min postapplication, CSMC tone decreased, but then the inhibitory effect of histamine was replaced by its stimulatory action (Fig. 1, *b*). On postapplication minutes 4-5, the tone of LN strips started to increase, restored to initial level, and on minutes 8-10 significantly overshot it. The long-term perfusion with histamine for 15-20 min increased frequency and amplitude of spontaneous phasic contractions. Pronounced chronoinotropic correlation was observed in 15-20% preparations: the increase in the rate of phasic contractions was accompanied by a decrement of their amplitude. At high concentration of 10^{-4} M, histamine stimulated CSMC contractile activity: it pronouncedly elevated the tonic stress and increased the rate and amplitude of phasic contractions (Fig. 1, *c*).

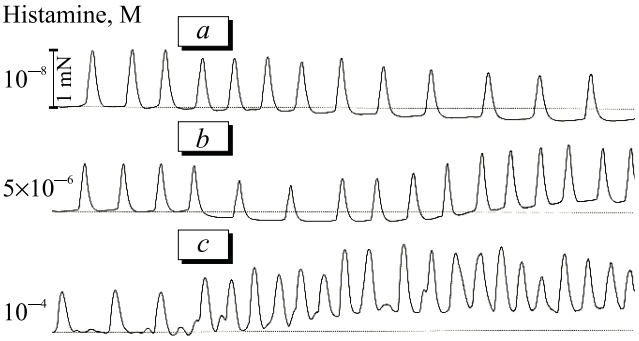

Fig. 1. Effect of histamine on contractile activity of CSMC in mesenteric lymph nodes. The horizontal baselines show the level of tonic force developed by the preparation in physiological saline.

Slow development of histamine effects on contractile activity of CSMC is noteworthy. It is known that in lymphatic vessels, histamine (10^{-7} M) markedly increases the frequency of phasic contractions and significantly decreases their amplitude as early as during 15-20 sec postapplication with the reaction maximum observed to the end of the first postapplication minute [9]. In contrast, in this study the effect of the same concentration of histamine started to develop only by the end of postapplication minute 3 with maximum of the reaction observed on minutes 10-15. Moreover, the range of the changes in parameters of contractile activity also fundamentally differed in LN and lymphatic vessels. While histamine (5×10^{-6} M) rapidly elevated the tonic stress by 200-220% in lymphatic vessels [7], similar rise in tonic stress of CSMC did not exceed 30% (Fig. 1, *b*). At high concentrations (10^{-5}-10^{-4} M) histamine markedly increased the tone of lymphatic vessel walls, which was usually accompanied by moderation of phasic contractile activity [7,8]. In our experiments, these concentrations of histamine increased only the amplitude of phasic contractions of CSMC with insignificant rise of their tone.

Application of histamine in a concentration of 10^{-6} M to CSMC preparation with preliminary blocked H_1-receptors with diphenhydramine (10^{-6} M) produced only an inhibitory effect on contractile activity. In contrast, preliminary block of H_2-receptors with cimetidine (10^{-6} M) markedly reduced (but did not completely eliminate) the inhibitory effects of histamine observed during the first postapplication minutes and potentiated its stimulatory action on 5-15 min postapplication (Fig. 2).

2-Pyridylethylamine, a specific agonist of H_1-receptors, produced a dose-dependent positive chrono- and inotropic effect on contractile activity of CSMC. In contrast, a specific agonist of H_2-receptors dimaprit dose-dependent by reducted both tonic stress and the rate and amplitude of phasic contractions. The stimulatory effect of 2-pyridylethylamine was prevented by preliminary application of diphenhydramine, while the inhibitory effect of dimaprit was prevented by cimetidine.

Thus, we can conclude that the effect of histamine on CSMC is mediated via activation of H_1- and H_2-receptors on CSMC membranes. At low concentrations, histamine predominantly stimulates H_2-receptors resulting in moderation of tonic stress and decrease in parameters of phasic contractile activity. At high concentrations, histamine activates H_1-receptors, which potentiate the contractile activity surpassing the inhibitory effect of activated H_2-receptors.

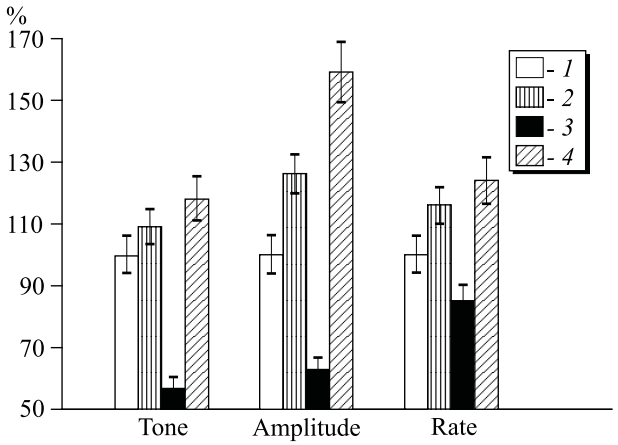

Fig. 2. Effect of histamine (10^{-6} M) on contractile activity of CSMC in mesenteric lymph nodes with preliminary blocked H_1- or H_2-receptors. The tone, amplitude, and the rate of phasic contractions in physiological saline were taken for 100%. *1*) control; *2*) histamine; *3*) histamine+diphenhydramine; *4*) histamine+cimetidine.

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