Effect of Acidosis on Contractile Function of Mesenterial Lymphatic Vessels in Bulls

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 7, pp. 16-19, July, 2001
Original article submitted March 16, 2001

Extracellular normocapnic acidosis inhibited spontaneous phasic contractile activity and reduced the tone of isolated bovine mesenterial lymphatic vessels. Acidosis induced dilation of lymphatic vessels due to activation of ATP-sensitive potassium channels in myocyte membrane and stimulation of NO production by endotheliocytes.

Key Words: *lymphatic vessels; acidosis; vasodilatation; ATP-sensitive potassium channels; nitric oxide*

Intensive physical work under conditions of insufficient blood supply is associated with tissue acidosis and dilation of blood vessels [3,4,11]. Mechanisms of vasodilatatory effect of acidosis are not completely understood. It was postulated that the decrease in smooth muscle cells (SMC) tone results from decreased intracellular calcium concentration [3,4,9,10]. At the same time, some authors report reduced sensitivity of regulatory and contractile proteins in vascular SMC to Ca^{2+} in acidosis [5]. Contractile activity of SMC in lymphatic vessels during acidosis has not been studied yet. At the same time, active transport via lymphatic vessels plays a key role in the development of pathological process in the tissue [1]. In light of this, the aim of the present study was to investigate the reaction of SMC in bovine mesenterial lymphatic vessels to low pH in the incubation medium.

MATERIALS AND METHODS

The experiments were carried out on isolated efferent bovine lymphatic vessels 1.5-2.5 mm in diameter (38 segments from 14 healthy animals) in order to exclude neurohumoral and local regulatory effects on their contractile function. Lymphatic vessels were isolated 10-15 min after exsanguination and delivered to the laboratory in a cold (2-4°C) Krebs solution. The experiments were conducted within 2-6 h after isolation of the material. Previous studies showed that spontaneous contractile activity of myocytes in isolated lymphatic vessels did not change significantly for 10-12 h [2]. The adipose and connective tissues surrounding the lymphatic vessels were removed under an MSSO-12 microscope and rings (3-4 mm wide, n=46) were cut [2]. In some specimens (n=17) the endothelium was mechanically removed, which allowed to exclude hyperpolarizing effect of endothelial NO. In all experiments, the initial stain calculated according to Laplase law corresponded to 8 cm transmural pressure H_2O [2]. The preparations were placed into a chamber perfused (1 ml/min) with Krebs solution (in mM: NaCl 120.4, KCl 5.9, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 5.5) saturated with gas mixture (95% O_2 and 5% CO_2) at 37°C. Contractile activity was recorded using a 6MX1C mechanoelectric transducer (in isometric regimen) coupled with a H-3031/3 writter. After stabilization of spontaneous contractile activity at pH 7.4, the preparations were exposed to pH 7.3, 7.2, 7.1, and 7.0. Normocapnic acidosis was modeled by adding HCl, pH was measured with an I-120 ionometer. The duration of incubation in low pH saline did not exceed 5 min, which allowed to exclude possible changes in intracellular pH [9]. For evaluation of the mechanisms underlying the effects

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0007-4888/01/1321-0622$25.00

of acidosis on myocytes in lymphatic vessels we used nonspecific potassium channels blocker $BaCl_2$ (10^{-4} M) and selective blocker of ATP-sensitive potassium channels glybenclamide (5×10^{-6} mol/liter, Sigma). The role of NO in acidosis-induced relaxation of SMC was studied on denuded vessels or by adding N^G-monomethyl-L-arginine (L-NMMA, 10^{-5} M, Calbiochem) to the incubation medium. The preparations were incubated for 20 min at pH 7.4 in the presence of one of these substances and then exposed to acidosis. The data were processed statistically using Student *t* test.

RESULTS

In all experiments spontaneous contractile activity with a mean phase contraction (PC) amplitude 7.60±0.63 mN and frequency 6.20±0.57 per min was recorded 10-15 min after the start of incubation in Krebs solution with pH 7.4. Decreasing pH of the incubation solution to 7.3 slightly decreased the frequency and amplitude of PC against the background of unchanged tone. Incubation at pH 7.2 significantly reduced PC frequency and amplitude after 1 min. In some experiments spontaneous activity disappeared for 30-40 sec but then was restored. On the 5th min of incubation at pH 7.2 the amplitude and frequency of PC were 3.90±0.41 mN and 2.70±0.34 per min, respectively. The tone decreased insignificantly (by 5.30±0.61% of initial level).

Incubation at pH 7.1 induced rapid changes in contractile activity of SMC in lymphatic vessels: PC frequency and amplitude markedly decreased after 10-15 sec. After 2-min incubation PC of SMC were stopped and the tone decreased. Maximum SMC relaxation (11.70±1.81% of the initial tone) was observed on min 4 of incubation at pH 7.1. Further increase in H^+ concentration (pH 7.0) led to rapid inhibition of spontaneous PC and drastic drop of the tone (by 21.4% of initial level, Fig. 1). In normal Krebs saline all parameters of contractile activity of lymphatic SMC returned to normal within several minutes. These data suggest that the reaction to acidosis in lymphatic SMC (similarly to blood vessel SMC [11]) is determined by H^+ concentration, depends on the dose, and is reversibe within pH range of 7.4-7.0.

Reactions of SMC in denuded lymphatic vessels to weak acidosis (pH 7.3) did not differ from those in intact preparations. Incubation at pH 7.2 decreased PC amplitude and frequency more slowly than in intact vessels: relaxation peaked on min 5 of incubation (4.20±0.66% of the initial tone). Incubation at pH 7.1 and 7.0 increased the relaxation rate and amplitude (Fig. 1). These data suggest that in mild acidosis the reaction of lymphatic vessels is determined by the response of SMC to increased H^+ concentration. Lower amplitude of relaxation of denuded lymphatic vessels at pH 7.0 shows that high H^+ concentration causes dilatation of lymphatic vessels both directly and via its action on endothelial cells.

Since the amplitude of relaxation in denuded vessels at pH 7.1 and 7.0 was lower than in intact vessels, in the next experimental series we studied the mechanisms underlying the effect of acidosis on endothelial cells. There are data that some factors can stimulate NO production by endotheliocytes of lymphatic vessels resulting in SMC relaxation via activation of soluble cytoplasmic guanylate cyclase [6]. In our experiments, pretreatment with NO synthesis blocker L-NMMA did not change the reaction of SMC in intact lymphatic vessels to weak and moderate acidosis. Incubation of L-NMMA-pretreated vessels at pH 7.1 and 7.0 inhibited spontaneous activity and decreased their tone (Fig. 1). These data suggest that mild acidosis did not change NO production in endotheliocytes. High H^+ concentration stimulates NO synthesis and release from endotheliocytes, which contributes to SMC dilatation.

Mild acidosis (pH 7.3 and 7.2) did not change contractile activity of SMC in intact vessels pretreated with $BaCl_2$ (n=5) and glybenclamide (n=10). Minor decrease in PC frequency was statistically insignificant. Incubation in $BaCl_2$- or glybenclamide-pretreated vessels at pH 7.1 decreased PC frequency and amplitude on the first minute of application by 21.30±2.72 and 35.30±4.18%, respectively. On minutes 3-4 phase activity was stopped and the tone decreased (Fig. 2). Incubation at pH 7.0 resulted in a more rapid inhibition of spontaneous activity and more pronounced

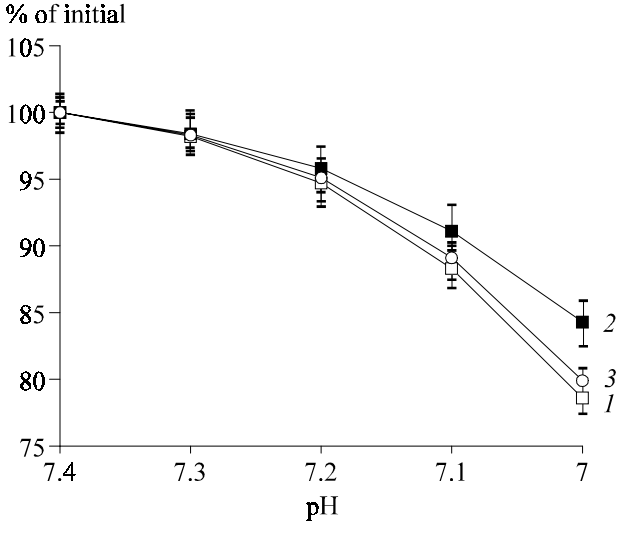

Fig. 1. Effect of extracellular acidosis on the tone of mesenterial lymphatic vessels. *1*) intact vessel, *2*) denuded vessel, *3*) intact vessel+N^G-monomethyl-L-arginine. Here and in Fig. 2: ordinate: tension, % of initial level. Initial level corresponds to 7.4 on abscissa.

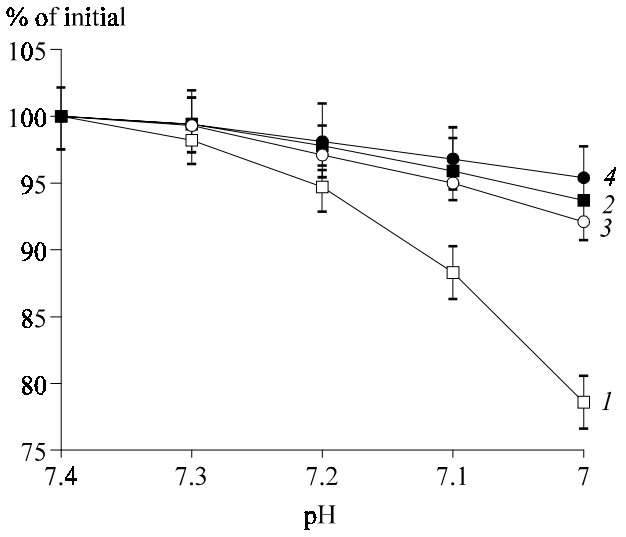

Fig. 2. Effect of potassium channel blockers, glybenclamide (*2, 4*) and $BaCl_2$ (*3*), on acidosis-induced relaxation of intact (*1, 2*) and denuded (*3, 4*) mesenterial lymphatic vessels. *1*) without blockers.

drop of SMC tone (by 6.30±0.76%). The effect of acidosis on denuded vessels pretreated with glybenclamide differed from that on intact vessels. This difference was most pronounced at pH 7.0 (Fig. 2).

Experiments with potassium channel blockers showed that acidosis-induced relaxation of lymphatic vessels is realized via opening of potassium channels in myocyte membrane. Since glybenclamide is a specific blocker of ATP-sensitive potassium channels, the absence of lymphatic vessel dilatation in response to mild acidosis and its significant attenuation at pH 7.0 suggest that ATP-sensitive potassium channels in SMC can be directly activated by H^+. Activation of ATP-sensitive potassium channels results in membrane hyperpolarization, closing of voltage-dependent Ca^{2+} channels, decrease of cytoplasmic calcium concentration, and relaxation of SMC. The role of ATP-sensitive potassium channels in acidosis-induced relaxation was previously shown on coronary arterioles and brain vessels [7,8].

Thus, SMC of lymphatic vessels are more resistant to acidosis than blood vessel SMC. Moderate acidosis only slightly decreased the tone and reduced the frequency and amplitude of spontaneous PC in lymphatic vessels without impairing their transport function. The effect of moderate acidosis is mediated by direct activation of ATP-sensitive channels in myocyte membranes. Further increase in extracellular H^+ concentration inhibits spontaneous contractions and cause pronounced vasodilatation due to opening of ATP-sensitive potassium channels in myocyte membranes and stimulation of NO production by endotheliocytes.

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