ISSN 0012-4966, Doklady Biological Sciences, 2019, Vol. 484, pp. 10–12. © Pleiades Publishing, Ltd., 2019.
Russian Text © G.I. Lobov, D.P. Dvoretskii, 2019, published in Doklady Akademii Nauk, 2019, Vol. 484, No. 5.

PHYSIOLOGY

Endothelium-dependent Hyperpolarization–Mediated Relaxation Pathway in Bovine Mesenteric Lymph Nodes

G. I. Lobov[a,*] and Corresponding Member of the RAS D. P. Dvoretskii[a]

Received September 17, 2018

Abstract—Endothelium-dependent relaxation mechanisms have been studied in phenylephrine-precontracted capsules of bovine mesenteric lymph nodes studied in vitro. Tetraethylammonium chloride and TRAM-34 in a solution with L-NAME and Indomethacin, which suppress the production NO and prostacyclin of endothelium, increased the tone of the lymph nodes. We believe that in bovine mesenteric lymph nodes, the dilation mechanism is mediated by hyperpolarization of the endothelium, which is associated with activation of large- and intermedium conductance Ca^{2+}-activated potassium channels.

DOI: 10.1134/S001249661901006X

Numerous reports published during the past 30 years suggest that vascular endothelium plays an important role in the arterial tone regulation [4, 5, 13]. Enhanced shear stress of the endothelium and a number of biologically active substances elicit the formation of vasoactive substances, such as nitric oxide (NO), prostacyclin (PGI_2), and endothelial hyperpolarizing factor (EDHF) [13]. NO and PGI_2 produced by endothelium diffuse to the vessel smooth muscle cells (SMCs), where they activate the intracellular signaling pathways leading to vasodilatation [6, 7]. Later, vasodilatation in response to acetylcholine (ACh), as well as shear stress, in the presence of NO synthase and cyclooxygenase were shown in some arteries; i.e., the third pathway of endothelium-dependent vasodilatation was found, which consisted in hyperpolarization of the SMC membrane [7]. The influence of the endothelium-derived substances (epoxyeicosatrienoic acids, hydrogen peroxide, hydrogen sulfide, etc.) on the SMC membrane leads to membrane hyperpolarization and myocyte relaxation [13]. Some evidence suggests that the endothelial cell hyperpolarization spreads to SMCs through the myoendothelial gap junctions [6, 7]. Later, the endothelium ability to evoke SMC hyperpolarization and relaxation was found in some veins [9, 15].

Along with the blood vessels, endothelial vasodilators, such as NO and PGI_2, were identified in the lymphatic vessel (LV) endothelium [10, 11, 14] and in that of lymph nodes (LNs) [1, 2]. Regarding the third mechanism of the endothelium-derived vasodilatation, via EDHF formation, there is no evidence so far that it also functions in LVs or LNs.

[a] *Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, 199034 Russia*

* *e-mail: gilobov@yandex.ru*

The goal of this study was to ascertain the mechanism of the LN capsule relaxation through endothelial hyperpolarization and to understand the role of this mechanism in the modulating effect of endothelium on the contractile function of LN SMCs.

The subject of our study was mesenteric LNs of 16- to 18-month-old bulls. Lymph nodes were isolated 15 min after bleeding the animals, placed into saline cooled to 2–4°C, and delivered into the laboratory. The capsule strips (8–10 mm long and 1 mm wide; $n = 36$) were cut out, while the subcapsular sinus was retained. In ten strips, the subcapsular sinus was removed. Then, the capsule strips were placed in a myograph chamber with a FORT 10 sensor (World Precision Instruments, United States). The experiments were performed under continuous supply of oxygenated physiological saline of the following composition, mM: NaCl, 120.4; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 15.5; and glucose, 11.5. The physiological saline was saturated with a gas mixture consisting of 95% of O_2 and 5% of CO_2 at a temperature of 37.0 ± 0.1°C. The technique was described earlier in detail [1]. The preparations were exposed to an initial tension of 0.45 mN/mm, which corresponded to a transmural pressure of 50 mm H_2O, and, after a 30-min stabilization, their contractile activity was recorded. The following agonists and antagonists were used: phenylephrine hydrochloride (Sigma-Aldrich, United States, 1×10^{-5} M); acetylcholine chloride (Sigma-Aldrich, 1×10^{-9} to 1×10^{-5} M); methyl ether Nώ-nitro-L-arginin (L-NAME, MP Biomedicals, United States, 1×10^{-4} M); indomethacin (Sigma-Aldrich, 1×10^{-5} M); tetramethylammonium chloride (TEA, Sigma-Aldrich, 1×10^{-3} M); apamin (Sigma-Aldrich, 1×10^{-7} M); TRAM-34 (1-

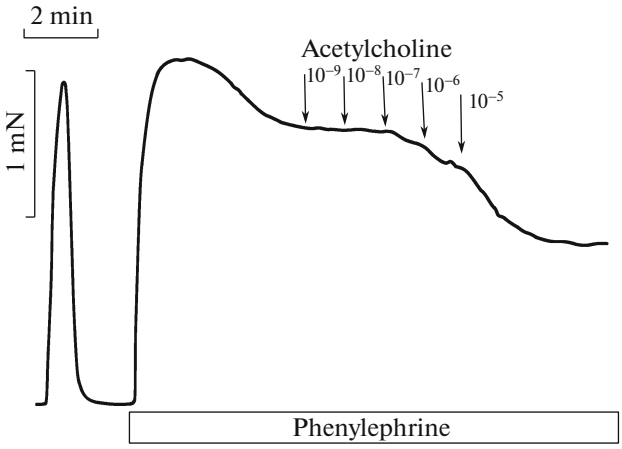

Fig. 1. The concentration-dependent effect of acetylcholine on the phenylephrine-precontracted bovine lymph-node capsule. On the left of the curve, a single spontaneous phase contraction of the LN capsule; *x* axis, concentration, M; *y* axis, tension.

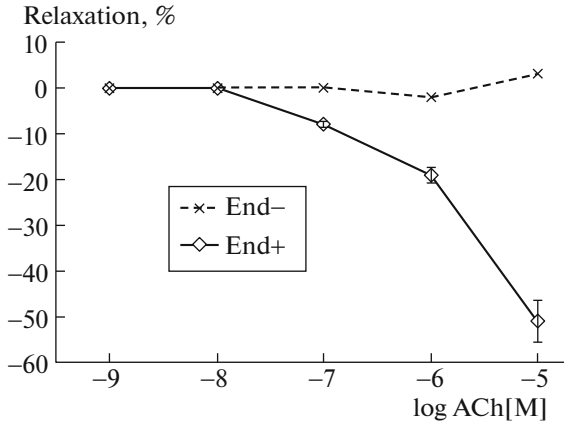

Fig. 2. Relaxation of the phenylephrine-precontracted (1×10^{-5} M) LN-capsule strips in response to acetylcholine added at concentrations of 1×10^{-9} to 1×10^{-5} M. End+, LN capsule with endothelium, $n = 12$; End–, deendothelized LN capsule, $n = 10$. Data ($M \pm m$) are shown in percents of the contraction amplitude in response to phenylephrine.

[(2-chlorphenyl) diphenylmethyl]-1H-pirasol, Sigma-Aldrich, 1×10^{-7} M).

The results were processed statistically using the Statistica 6.1.478 software (StatSoft, United States). Significant differences were determined using Student's *t* test. Differences were considered statistically significant at $p \le 0.05$.

Rhythmical contractions of LN capsule strips (34 out of 36) were observed 30 min after the strips were incubated in saline. The parameters of these phase contractions were described earlier [1]. Addition of phenylephrine to the solution with LN capsule strips led to a rapid increase in tonic tension, which stabilized at a new level after 2–3 min. Afterwards, increasing ACh concentrations were added (1×10^{-9} to 1×10^{-5} M), and the LN strip relaxation was estimated (Fig. 1). At concentrations of 1×10^{-9} and 1×10^{-8} M, acetylcholine had no effect on the LN strip tension. With increasing ACh concentration up to 1×10^{-6} M, the tension of strip preparations reduced, and, at a concentration of 1×10^{-5} M, ACh caused the maximum relaxation reaching 51.4 ± 4.6% of the contraction induced by phenylephrine (Fig. 1). The preparation response to the ACh concentration of 1×10^{-4} M did not differ significantly from that at the ACh concentration of 1×10^{-5} M. The deendothelized strips of LN capsules did not respond to the aforementioned ACh concentrations. The tension of the deendothelized strips remained unchanged in response to ACh, which indicates that the LN capsule relaxation caused by ACh is endothelium-dependent. Figure 2 shows the averaged results of these experiments.

In subsequent experiments, the following substances were added 20 min before exposure to ACh: L-NAME (an NO synthase inhibitor), indomethacin (Indo, a cyclooxygenase inhibitor), and specific blockers of K^+ channels at different combinations: TEA (a blocker of Ca^{2+}-activated K^+ channels of a high conductivity), TRAM-34 (a blocker of Ca^{2+}-activated K^+ channels of an intermediate conductivity) and apamin (a blocker of Ca^{2+}-activated K^+ channels of a low conductivity). Figure 3 shows the results of this experimental series. In a solution with L-NAME, relaxation of the phenylephrin-precontracted LN capsule was significantly suppressed (down to 34.2 ± 2.9%), which proves the important role of NO in the relaxing effect of ACh on the strips of the LN capsule. Similar results were obtained earlier in studying NO-dependent regulation of the LN-capsule contractile function [1, 2]. To assess the role of PGI_2 in ACh-induced relaxation of the LN capsule, the capsule was treated with two inhibitors (L-NAME + Indo). In this case, the ACh-evoked relaxation of the LN capsule was less than the effect of L-NAME alone (28.3 ± 2.4%), but the difference was insignificant, which suggests that PGI_2 role in the LN capsule relaxation was not of great importance.

Despite the inhibition of NO and PGI_2 production by endotheliocytes, ACh still caused a significant relaxation of the LN capsule contracted after the effect of phenylephrine. This suggested the existence of an additional signaling pathway of LN capsule relaxation. To date, such a pathway is well identified in arteries and veins and is known to be related to the endothelium-dependent hyperpolarization triggered by Ca^{2+}-activated K^+ channels [7, 9]. At the first stage of possible involvement of the Ca^{2+}-activated K^+ channels in the LN capsule relaxation, we applied TEA and recorded a slight decrease in ACh-evoked relaxation (Fig. 3). The level of relaxation was on the border of statistical significance. Some published evidence suggests that, under physiological conditions, endothelial

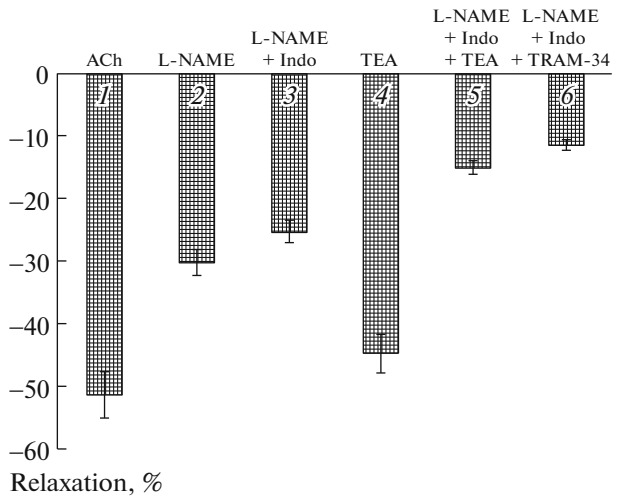

Fig. 3. The effects of NO-synthase and cyclooxygenase inhibitors (L-NAME and Indo, respectively) and specific blockers of the Ca^{2+}-activated K^+ channels (TEA and TRAM-34) on the ACh-evoked relaxation of the LN-capsule strips. *1,* ACh; *2,* L-NAME; *3,* Indo; *4,* TEA. Against the background of the L-NAME + Indo effect and the ACh-evoked relaxation, either TEA (*5*) or TRAM-34 (*6*) was added into the solution.

vasodilators are often formed in excessive amounts and can influence either production or the effects of other dilators. For instance, when studying the coronary microcirculation in dogs, the exogenous NO was found to inhibit either the production or the effect of EDHF [9]. It was also found that, in Sprague Dawley rats, both endogenous and exogenous NO reduced significantly the EDHF-mediated depressor response [8]. Taking into account these data, we studied the TEA effect in a solution containing L-NAME + Indo. In these experiments, a significant decrease was observed in the ACh-evoked relaxation of LN capsule strips as compared to their relaxation in the solution containing only L-NAME + Indo (the relaxation amplitude was 15.1 ± 1.9% of the phenylephrine-caused contraction amplitude versus 25.3 ± 3.1% in the solution with L-NAME + Indo, Fig. 3).

Suppression of the ACh-evoked LN capsule relaxation was even stronger after addition of TRAM-34 into the solution containing L-NAME + Indo (the relaxation amplitude was 11.4 ± 1.52% of the phenylephrine-evoked contraction amplitude, Fig. 3). Addition of apamin into the solution containing L-NAME + Indo caused no significant changes in the ACh-evoked relaxation of the LN capsule (data not shown).

Published evidence indicates that the effects of vasodilators (NO, PGI_2, and EDHF) differ significantly in various vascular regions in animals of different species [3, 13]. In some reports (e.g., [12]), NO was found to be the main endothelium-produced vasodilator in large arteries, while in small arteries, vasodilatation is mainly EDHF-mediated. The chemical nature of EDHF has not been determined so far, but it is universally recognized that the end result of the EDHF effect is opening of the Ca^{2+}-activated K^+ channels, which is followed by membrane hyperpolarization of the vascular SMCs and their relaxation.

The results of our study indicate that, in the bovine mesenteric LN, the endothelium-dependent relaxation mechanism mediated by endothelial hyperpolarization is activated along with NO- and PGI_2-mediated mechanisms. Since the ACh-evoked relaxation was significantly reduced after TEA and TRAM-34 application, we suggest that the EDHF-mediated relaxation mechanism in the LN capsule is triggered via activation of Ca^{2+}-activated K^+ channels of high and intermediate conductivities.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Lobov, G.I. and Pan'kova, M.N., *Ross. Fiziol. Zhu. im. I.M. Sechenova,* 2012, vol. 98, no. 11, pp. 1350–1361.
2. Unt, D.V. and Lobov, G.I., *Byull. Eksp. Biol. Med.,* 2017, vol. 164, no. 8, pp. 145–149.
3. Brandes, R.P., Schmitz-Winnenthal, F.H., Feletou, M., et al., *Proc. Natl. Acad. Sci. U.S.A.,* 2000, vol. 97, no. 17, pp. 9747–9752.
4. Furchgott, R.F. and Zawadzki, J.V., *Nature,* 1980, vol. 288, no. 5789, pp. 373–376.
5. Furchgott, R.F., Cherry, P.D., Zawadzki, J.V., and Jothianandan, D., *J. Cardiovasc. Pharmacol.,* 1984, vol. 6, suppl. 2, pp. S336– S343.
6. Hurjui, L., Serban, I.L., Oprişa C., et al, *Rev. Med. Chir. Soc. Med. Nat. Iasi.,* 2011, vol. 115, no. 1, pp. 168–170.
7. Jin, X., Satoh-Otonashi, Y., Zamami, Y., et al., *J. Pharmacol. Sci.,* 2011, vol. 116, no. 4, pp. 332–336.
8. Kobuchi, S., Miura, K., Iwao, H., and Ayajiki, K., *Eur. J. Pharmacol.,* 2015, vol. 762, pp. 26–34.
9. Liu, Z.G., Ge, Z.D., and He, G.W., *Circulation,* 2000, vol. 102, no. 19, suppl. 3, pp. 296–301.
10. Nishikawa, Y., Stepp, D.W., and Chilian, W.M., *Am. J. Physiol. Heart Circ. Physiol.,* 2000, vol. 279, no. 2, pp. H459–H465.
11. Reeder, L.B., Yang, L.H., and Ferguson, M.K., J. Surg. Res., 1994, vol. 56, no. 6, pp. 620–625.
12. Shimokawa, H., Yasutake, H., Fujii, K.J., et al., *Cardiovasc. Pharmacol.,* 1996, vol. 28, pp. 703–711.
13. Vanhoutte, P.M., Shimokawa, H., Tang, E.H., and Feletou, M., *Acta Physiol.,* (Oxford), 2009, vol. 196, no. 2, pp. 193–222.
14. Yokoyama, S. and Ohhashi, T., *Am. J. Physiol.,* 1993, vol. 264, no. 5, part 2, pp. H1460–H1464.
15. Zhang, R.Z., Yang, Q., Yim, A.P., et al., *Vascul. Pharmacol.,* 2006, vol. 44, no. 3, pp. 183–191.

Translated by A. Nikolaeva