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COMMENTARY

MECHANISMS FOR REGULATING TONE IN LYMPHATIC VESSELS

TOSHIO OHHASHI*
The 1st Department of Physiology, Shinshu University School of Medicine, Matsumoto 390, Japan

The lymphatic system serves a major bodily function, which is to provide a fluid return system from the tissues, restoring to the blood circulation both fluid and other substances, especially plasma protein that leaks out of the capillaries and cannot be reabsorbed directly back into the circulation. Thus, the lymphatic system plays an important role in the overall homeostasis of body fluids. The mechanisms by which these functions are carried out depend on active and passive driving forces, as well as upon the rate of lymph production in organs and tissues. The active driving force is due to the intrinsic contractility of the lymph vessels [1–5]. In bovine mesenteric lymphatics in which smooth muscles are well-developed in the wall [6], lymphatic smooth muscle tone seems to play a major role in the regulation of spontaneous contractions, thereby significantly affecting the active driving mechanism [4, 5].

The passive driving mechanisms are due to the compression and suction of lymph vessels resulting from extrinsic activities such as the muscle pump [7, 8], the respiratory movement [9], and arterial pulsation [10, 11]. In dog thoracic duct which contains a few smooth muscle layers in the wall [12], passive driving forces such as oscillatory changes of intrathoracic pressure are believed to be main mechanisms of lymph propulsion in the vessel. In dog thoracic duct, lymphatic smooth muscle tone seems to play a major role in the elastic behavior of the wall, thereby affecting the passive driving mechanisms.

Thus mechanisms for humoral regulation of the lymphatic smooth muscle tone in two different kinds of lymph vessels, bovine mesenteric lymphatics and dog thoracic ducts, will be reviewed in this commentary.

Spontaneous contractions of lymphatic smooth muscles

It has been suggested that spontaneous contraction of lymphatic smooth muscles is the major factor responsible for lymph propulsion under physiological conditions [1–5]. Hargens and Zweifach [13] investigated spontaneous contractions of mesenteric lymph vessels in rats and guinea pigs and came to the conclusion that lymphatic wall tension correlated well with frequency of the contractions, suggesting a myogenic origin for the contraction mechanism. The vibratory stimulation produced spontaneous contractions in quiescent preparations isolated from bovine mesenteric lymph vessels [5]. An acceleration of the rhythm of existing spontaneous contractions was also brought about by the vibratory stimulation [5]. Similar findings have been obtained in isolated dog portal vein and ureter [14]. These results suggest that the spontaneous contraction of lymphatic smooth muscles is regulated not only by the magnitude of stretch but also by the rate and acceleration of deformation in the lymphatic vessel wall. In brief, active lymph transport generated by the spontaneous contractions may be controlled by the rate and volume of lymph flow itself.

Smooth muscles in the bovine mesenteric lymph vessels are well developed and arranged in the following three layers: the internal longitudinal, intermediate circumferential, and external longitudinal. The outer layer is much thicker than the other two. A large number of mitochondria, gathered in a cluster, are seen on both sides of the nucleus in the smooth muscle cells. Numerous glycogen granules are found among and around the mitochondria [6]. These structural features may be a morphological manifestation of the high metabolic activity required for spontaneous contractions of the lymphatic smooth muscles. A number of blood capillaries and nonmyelinated nerve fibers are found within the smooth muscle layers as well as in the adventitia [6]. The presence of vasa vasorum within the medium may reflect a relatively high oxygen requirement of the lymphatic smooth muscle cells and the relatively low oxygen supply from the lymph flow within the lymph vessel. An ample supply of oxygen will be required to maintain spontaneous contractions in the lymph vessel.

We have investigated the electrical activity of the lymphatic smooth muscles by the use of the sucrose gap [15] and intracellular electrode techniques [16, 17]. The mean resting membrane potential of the smooth muscle cells in bovine mesenteric lymph vessels is about −50 mV. This value is compatible with the maximum diastolic depolarization in the same lymph vessel reported by Ward *et al.* [18]. The resting potential sometimes shows rhythmic fluctuations or slow waves which resemble that in visceral smooth muscles [19]. The resting potential seems to be lower in the lymphatic smooth muscle with spontaneous contractions than that without the contractions. The minimum depolarization necessary for inducing contractions is about 6 mV in the

* Correspondence: Toshio Ohhashi, M.D., Ph.D., The 1st Department of Physiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390, Japan. Tel. 0263-35-4600 (Ext. 5170); FAX 0263-36-5149.

lymphatic smooth muscle cell [17]. In potassium-free solution, the resting membrane is depolarized by about 9 mV, and the lymphatic smooth muscle develops a sustained tension. Ouabain at 10^{-5} M also causes a depolarization of the membrane potential and a sustained contraction in bovine mesenteric lymph vessels. These observations suggest that changes of membrane potential may play a major role in the activation of the contractile protein in the lymphatic smooth muscles and also that there is an electrogenic sodium pump in the plasma membrane. The depolarization and tension development in potassium-free solution may be due to a decreased activity of the electrogenic sodium pump.

The firing of lymphatic action potential could be classified in two patterns, i.e. (1) short trains consisting of several spikes, and (2) single spikes or irregular spike discharges [16]. Occasionally the action potentials are superimposed upon the rising phase of the slow fluctuations [16]. Calcium current may play a major role in producing the spike discharge in bovine lymphatic smooth muscles [15].

Humoral regulation of smooth muscle tone in bovine mesenteric lymphatics

Tonic contractions of smooth muscles in bovine mesenteric lymph vessels have been induced by bradykinin (BK)* [20], 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), norepinephrine (NE), histamine, dopamine, and acetylcholine (ACh). The decreasing order of potency in the contractile responses was as follows: BK > 5-HT > $PGF_{2\alpha}$ > NE > histamine > dopamine > ACh [21]. The smooth muscles were particularly sensitive to BK and 5-HT. 5-HT produces contraction of the isolated bovine mesenteric lymph vessels, which is mainly mediated via activation of the 5-HT_2 receptors [22, †]. Pharmacological subtypes of postjunctional α-adrenoceptors have been characterized in bovine mesenteric lymph vessels [23]. We determined the pA_2 values, i.e. negative logarithm of a dissociation constant of the antagonist, of yohimbine and prazosin in the lymph vessels. The pA_2 value of yohimbine against phenylephrine was markedly different from that against clonidine. This observation suggests that α-adrenergic receptors in the lymphatic smooth muscle cells are not uniform in subtype. The pA_2 value of yohimbine against clonidine was 7.6 in the lymph vessel. This value is very close to the pA_2 value of the antagonist to clonidine in rat aorta, in which receptors with high affinity to yohimbine have been observed [24]. This suggests that α_2-adrenoceptors are present in bovine mesenteric lymph vessels. The pA_2 value of prazosin against phenylephrine was also determined to be 7.2 in the lymph vessels. This may also indicate that the presence of α_1-adrenoceptors is defined in the lymphatic smooth muscle cells, because the pA_2 value obtained was compatible with values reported in the dog vascular smooth muscles, which had been confirmed to contain mainly α_1-adrenoceptors [25].

BK has been known to be related closely to the initiation and development of inflammation. It is a substance which raises the permeability of capillaries and possibly also of venules in the irritated area, thereby increasing the rate of production of interstitial fluid and lymph [26]. In bovine mesenteric lymph vessels, BK caused an acceleration in the rhythmicity of spontaneous contractions at low concentrations (10^{-11} M–10^{-10} M) and elicited dose-related contractions at high concentrations (>10^{-8} M) [20]. The threshold concentration for BK was 1/100th–1/500th of that for 5-HT. This high sensitivity to BK may be characteristic of the lymphatic smooth muscle. The BK-induced contractions may be produced by an increased Ca^{2+} influx through the plasma membrane and release of the membrane-bound and intracellular stored Ca^{2+}. Thus, the lymphatic smooth muscle cell may have three kinds of sources of activator Ca^{2+}: potential-dependent Ca^{2+} channel, receptor-operated Ca^{2+} channel, and 1,4,5 IP_3-sensitive Ca^{2+} channel. The increased uptake of Ca^{2+} into the BK-sensitive intracellular store may also contribute to the relaxing effect of β-agonists in the lymphatic smooth muscle cell [20].

On the other hand, vasoactive intestinal polypeptide (VIP), atrial natriuretic peptide (ANP), prostaglandin I_2 (PGI_2), prostaglandin E_2 (PGE_2), isoproterenol (ISP), 5-HT, adenosine, and adenine nucleotides cause relaxation in isolated bovine mesenteric lymph vessels precontracted by vasoactive substances [21, 27–30, †]. The lymph vessels are innervated by nerve fibers containing VIP [27]. Furthermore, VIP produced a marked relaxation of the isolated lymph vessels precontracted by 10^{-7} M BK. The magnitude of the maximum relaxation by 3×10^{-7} M VIP was significantly larger than those induced by the other vasodilative peptide hormones, neurotensin, substance P, methionine-enkephalin, and leucine-enkephalin. The minimum effective concentration of VIP in eliciting relaxations (~10^{-9} M) is significantly lower than those of ISP examined under similar conditions [21, 27]. Pharmacological studies using transmural stimulation have also suggested that a non-adrenergic and non-cholinergic inhibitory innervation may be present in bovine mesenteric lymph vessels [31]. Thus, the presence of VIP-containing nerve fibers, together with the demonstration of its potent lymphatic action, provides evidence that VIP may be a non-adrenergic and non-cholinergic inhibitory transmitter in the lymph vessel. Recently, a variety of polypeptides have been suggested as transmitter candidates of non-adrenergic and non-cholinergic inhibitory nerves in the cerebral arteries [32], and portal vein [33]. Some of the peptides coexist with classical transmitters [34]. Among these, particular attention is currently being paid to VIP as a putative transmitter, which mediates relaxations of cerebroarterial smooth muscle, as this peptide is a powerful vasodilator substance *in vitro* and *in vivo* [35]. A recent study also suggests that the relaxation induced by the activation of non-adrenergic and non-

* Abbreviations: BK, bradykinin; 5-HT, 5-hydroxytryptamine; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; NE, norepinephrine; ACh, acetylcholine; VIP, vasoactive intestinal polypeptide; PGE_2, prostaglandin E_2; ISP, isoproterenol; and ANP, atrial natriuretic peptide.

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cholinergic inhibitory neurotransmission in the rat stomach consists of at least two components to which VIP and nitric oxide contribute, respectively [36].

ANP is a newly discovered hormone secreted by the atria of the heart that has potent natriuretic and diuretic properties [37]. Its physiological role is believed to be that of protecting the organism against volume overload as suggested by a rise in plasma ANP with acute or chronic volume expansion [38]. Therefore, we investigated the mode of action of ANP on bovine mesenteric lymph vessels to elucidate the possible significance of ANP in lymph transport [29]. A low concentration of ANP (5 ng/mL) seems to inhibit lymph transport through a marked relaxation of lymphatic smooth muscles in the lymph vessels, which may play in part a compensatory role of keeping tissue fluid in interstitial spaces after a marked imbalance of body fluids produced by an increased release of ANP. The threshold concentration for ANP was about the same as that for VIP, being considerably lower than that of ISP examined under similar conditions [21]. The ANP-induced relaxation may be related, in part, to an increase in the level of guanosine 3′,5′-cyclic monophosphate, independent of the lymphatic endothelium.

5-Carboxamidotryptamine or 5-HT also produces a marked relaxation of the precontracted bovine mesenteric lymph vessels through the activation of the putative $5HT_4$ receptor [22, *].

Recently, a mixture of both β_1- and β_2-adrenoceptors has been demonstrated in various vascular preparations [39]. It may be reasonable to expect that both β_1- and β_2-adrenoceptors can be observed in the bovine mesenteric lymph vessels because the vessels have vein- and heart-like mechanical properties [5]. Isoproterenol, denopamine, and procaterol caused dose-dependent relaxations in the isolated lymph vessels precontracted by 10^{-6} M 5-HT [30]. There was no significant difference in the relaxant responses to the β-adrenoceptor agonists between the lymphatic preparations with and without the endothelium. Schild plot analyses showed that the slope and pA_2 values for metoprolol against denopamine were 1.10 and 7.59, respectively, and that those for ICI 118,551 against procaterol were 0.91 and 9.96. These results suggest that both β_1- and β_2-adrenoceptors are located on the smooth muscle cells in bovine mesenteric lymph vessels and that stimulation of either receptor produces a marked relaxation [30]. A question may be asked as to whether the different β-adrenoceptors play different roles in the mechanical properties of the lymph vessels or not. The present study showed that the pA_2 values for β_1- and β_2-adrenoceptors antagonists were 7.59 and 9.96, respectively, suggesting that the β_2-adrenoceptors in the lymph vessels may play an important role in the regulation of the lymphatic smooth muscle tone. Our previous study has also demonstrated that the β_2-adrenoceptors seem to have a regulatory role in the spontaneous contractions in the lymphatic preparations [40]. The physiological significance of the β_1-adrenoceptors in the lymph vessels is still unknown. The bovine mesenteric lymph vessels are innervated with β-adrenergic inhibitory nerve fibers [31]. The norepinephrine-containing nerve fibers are also observed histochemically in the lymph vessels [41]. The chemical transmitter of the inhibitory nerve fibers, norepinephrine, is well known to have a more marked affinity for β_1- than for β_2-adrenoceptors. These findings may show that one of the physiological roles of the β_1-adrenoceptors in the lymph vessels is related to the β-adrenergic inhibitory innervation.

Humoral regulation of spontaneous contractions in bovine mesenteric lymphatics

The present study was undertaken to investigate quantitatively the effects of vasoactive substances on spontaneous contractions in the isolated bovine mesenteric lymph vessel for elucidating the possible significance of the substances in the active lymph transport mechanism. An acceleration of the contraction rhythm took place following the administration of 10^{-7} M NE and 10^{-7} M $PGF_{2\alpha}$. After treatment with 10^{-6} M phentolamine, the administration of NE decelerated the rhythm and suppressed the amplitude of spontaneous contractions [28]. The negative chrono- and inotropic effects were antagonized by a β-adrenergic blocking agent, propranolol. Figure 1 summarizes the chronotropic effects induced by agonists on spontaneous contractions in the lymph vessels. The decreasing order of potency in the positive chronotropic effects is as follows: BK $\gg$ 5-HT $>$ $PGF_{2\alpha}$ $>$ NE $>$ histamine. The threshold concentration for BK was less than 10^{-11} M. The threshold concentration for 5-HT was 100–500 times greater than that for BK. The positive chronotropic effect induced by histamine was blocked completely by treatment with an H_1-antagonist, diphenhydramine [42].

In the lower half of Fig. 1 is shown the decreasing order of potency in the negative chronotropic effects of VIP, ANP, ISP, and histamine in the isolated bovine mesenteric lymph vessels. The ISP- and histamine-induced negative chronotropic effects were antagonized by treatment with β-adrenoceptor and H_2 antagonists, propranolol and cimetidine, respectively [42, 43]. PGI_2 and PGE_2 caused dose-related reductions in the amplitude of spontaneous contractions but no change of the rhythm of spontaneous contractions in the lymph vessels [28]. These findings suggest that BK, 5-HT, $PGF_{2\alpha}$, NE, and histamine in low concentrations may facilitate lymph flow through the lymph vessels, since the spontaneous contractions of the lymphatic smooth muscles may drive lymph centripetally due to the presence of unidirectional valves. On the other hand, VIP, ANP, ISP, PGI_2, and PGE_2 may produce inhibition of the active transport mechanism generated by the spontaneous contractions.

Humoral regulation of smooth muscle tone in dog thoracic duct

Contractions of the lymphatic smooth muscles in isolated dog thoracic ducts were induced by epinephrine, NE, 5-HT, histamine, and $PGF_{2\alpha}$ in a dose-dependent manner [44]. The decreasing order

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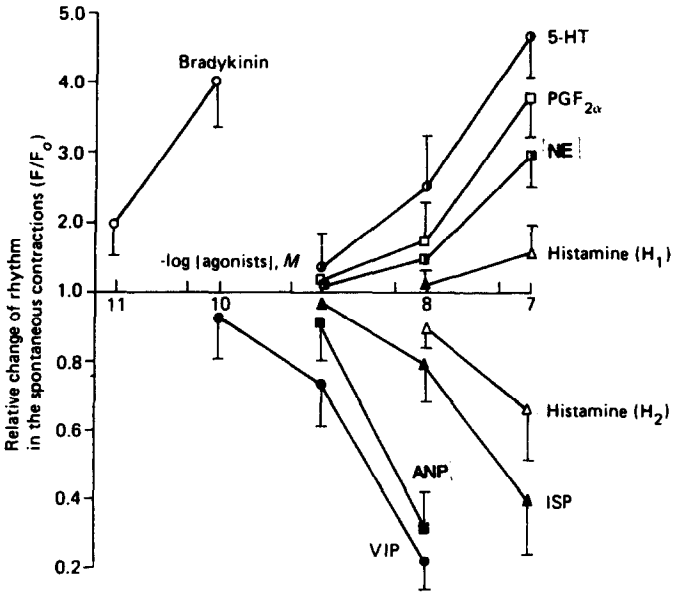

Fig. 1. Relationships between relative changes in the rhythm of spontaneous contractions in isolated bovine mesenteric lymph vessels and concentration of the vasoactive substances on a logarithmic scale. Abbreviations: 5-HT, 5-hydroxytryptamine; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; NE, norepinephrine; VIP, vasoactive intestinal polypeptide; ANP, atrial natriuretic peptide; and ISP, isoproterenol. The extent of changes in the rhythmicity (ordinate) is expressed in terms of F/F_0, where F and F_0 are the average frequency of spontaneous contractions during and before stimulation, respectively. Reprinted with permission from Ref. 43. Copyright (1987) S. Karger AG, Basel, Switzerland.

of potency in the contractile responses was as follows: epinephrine > NE > 5-HT ≫ histamine = $PGF_{2\alpha}$. There were no significant regional differences in the responses to the vasoactive agents. The contracting action of epinephrine and NE showed that activation of the α-adrenergic receptors may be involved in the modulation of smooth muscle tone, modifying the stressed and unstressed volume in dog thoracic duct. This is compatible with previous experimental findings obtained with isolated dog thoracic ducts [45, 46]. The catecholamine-induced contractions may be related, in part, to the histochemical and electron microscopic findings which demonstrate dense innervation by aminergic nerve fibers in the thoracic ducts [12]. Both α_1- and α_2-adrenoceptors are located on the lymphatic smooth muscles of dog thoracic ducts.

On the other hand, ACh, ISP, histamine, adenosine, and ATP caused dose-dependent relaxations in dog thoracic ducts precontracted by 10^{-5} M NE. The decreasing order of potency in the relaxant responses was as follows: ACh > ISP ≫ adenosine = histamine = ATP. There were no significant regional differences in the relaxant responses to the agents. Both β_1- and β_2-adrenoceptors are involved in the ISP-induced relaxation in the dog thoracic ducts.

ACh induced the most prominent relaxation response in the isolated dog thoracic ducts precontracted by NE. The previous studies, however, have demonstrated that ACh produced contractions only in the lymph vessels [45, 46]. A possible reason for the above discrepancy may concern the use of precontraction, the concentration of ACh, and mechanical damage of the endothelial cells. Actually, removal of the endothelium caused a complete inhibition of the ACh-induced relaxations in the precontracted dog thoracic ducts [47]. ACh, which failed to relax precontraction of the ring preparation when mounted separately, induced relaxation in the same preparation when it was mounted as a "sandwich" with the longitudinal strip. Pretreatment with atropine inhibited the ACh-induced relaxation in a competitive manner. The Schild plot showed a slope of 1.1 and a pA_2 value of 10.4. The ACh-induced relaxations in the lymphatic preparations with the endothelium were suppressed or abolished by pretreatment with oxyhemoglobin, methylene blue, and N^G-monomethyl-L-arginine, but the relaxations were unaffected by aspirin. It may be concluded that ACh-induced relaxation in dog thoracic ducts precontracted with NE is mediated by high-affinity muscarinic receptors in the lymphatic endothelial cells. Also, stimulation of the endothelial muscarinic receptors liberates a transferable endothelium-derived relaxing factor, which results in the relaxation of the lymphatic smooth muscles via the accumulation of cellular guanosine 3′,5′-cyclic monophosphate. Thus, the ACh-induced endothelium-dependent relaxation plays an important role in regulation of elastic behavior of the lymphatic wall, affecting passive driving forces in the lymph transport in dog thoracic ducts.

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