Lymphology 12 (1979) 4–6

Electrical Activity and Ultrastructure of Bovine Mesenteric Lymphatics

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Summary

Membrane action potentials of bovine mesenteric lymphatics were recorded simultaneously with isometric contractions by use of the sucrose gap method. The action potentials always had one-to-one correspondence to the contraction waves. From the effects of tetrodotoxin, manganese, cal-calcium-free enviroment, and barium chloride on the spontaneous contractions, it was suggested that calcium current may probably play a major role in producing spike discharge in the smooth muscles. Blood capillaries were found within the smooth muscle layers as well as in the adventitia.

Introduction

In lower animals, particularly in amphibia, the flow of lymph is maintained by rhythmically beating lymph hearts. Although there are no lymph hearts in the mammal, all lymphatics other than terminal capillary networks contain smooth muscles in their wall (1). It has been noted that mammalian lymphatics, at least in certain species and in certain areas, showed spontaneous active contractions which were capable of promoting lymph propulsion (1). Recently, the present authors have succeeded in recording membrane action potentials of lymphatic smooth muscles by means of the microelectrode technique, elucidated the mechanism of lymph propulsion, and examined the responses of lymphatics to several physiological vasoactive substances (2). These results will be published elsewhere.

The present experiments were undertaken to study spontaneous electrical activity in bovine mesenteric lymphatics by means of the sucrose gap technique. The motor activity of lymphatic smooth muscles was also investigated from the view point of ultrastructure.

Material and Methods

Segments of mesenteric lymphatics, between 0.5 and 3 mm in outer diameter, were dissected from fresh mesenterics of recently slaughtered cattle. Longitudinal strips, about 20 mm long and 0.5 mm wide, were cut from these segments. Each strip was mounted in a sucrose gap apparatus for simultaneous recording of electrical and mechanical activities. One end of the mounted strip was connected to a force-displacement transducer (Shinko-Tsushin UL-2) to record mechanical activity isometrically. This part of the strip was continuously superfused with the modified Locke's solution of the following composition in m mole/liter: NaCl 154.0, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 8.0, glucose 5.5. Another end was anchored at a fixed point and depolarized by the K_2SO_4 Locke's solution, which had the composition (m mole/liter): K_2SO_4 126.0, KCl 5.6, $CaCl_2$ 2.2, $KHCO_3$ 8.0, glucose 5.5. The middle section of the strip was superfused with an isotonic sucrose solution. Nonpolarizing Ag-AgCl wire electrodes, which were connected to a high input resistance preamplifier (Nihon Koden MEZ 8101), were placed in the conducting solution bathing each end of the strip. The outputs from the electrodes and the mechanoelectric transducer were recorded by a direct writing oscillograph (Sanei Sokki 8S). The lymphatic segments were fixed in a 4 % glutaraldehyde solution. Small pieces were then cut for embedding. After fixation in osmium tetroxide and dehydration in alcohol, the tissue blocks were embedded in epon. All sections were cut with the LKB Ultrotome, and the electron microscopy was done with the Hitachi HU-11A. The sections were stained double with lead citrate and uranyl acetate.

0024–7766 1300–0004 $ 02.00

Results and Discussion

Figure 1 demonstrates membrane action potential, of bovine mesenteric lymphatics recorded simultaneously with isometric contraction waves. The action potentials, which were about 3 sec in duration and similar in appearance to the pacemaker potentials recorded from some other smooth muscles, had one-to-one correspondence to the contraction waves. Each action potential preceded the corresponding spontaneous contraction wave by about 750 msec. The action potential could be divided into the following three phases: initial slow depolarization, spike and final slow repolarization. The average value of resting potential in lymphatic smooth muscles was estimated to be -33 ± 4 mV. The application of noradrenaline elicited a slight but long-lasting depolarization superimposed by frequent discharges of action potentials. The administration of acetylcholine in a relatively high concentration induced a transient depolarization followed by the occurrence of action potentials. Based on the effects of tetrodotoxin, manganese, calcium-free environment and barium chloride on the spontaneous activity, it was suggested that calcium current may play a major role in producing spike discharge in bovine lymphatic smooth muscles (3).

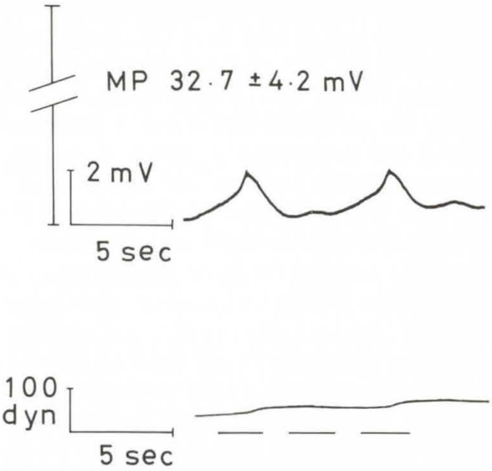

Fig. 1 Spontaneous electrical and mechanical activities of bovine mesenteric lymphatics recorded by means of the sucrose gap technique. Upper and lower tracings are action potentials and isometric phasic contractions, respectively. Broken lines indicate zero tension level.

Figure 2 represents a blood capillary found within the media of bovine mesenteric lymphatics. It was identified as a blood capillary by the presence of basement membrane and pericyte. An erythrocyte was also found in the lumen.

Fig. 2 Transverse section of a blood capillary found within the internal longitudinal smooth muscle layers (x5000)

The present electron microscopic observation revealed that blood capillaries, along with collagen fibers, entered deep into the well-developed smooth muscle layers of bovine mesenteric lymphatics. The distribution of blood capillaries in lymphatic walls may be much more widespread than has so far been believed. The presence of vasa vasorum within the media may reflect the relatively high oxygen requirements of the lymphatic smooth muscles and the relatively low oxygen supply from the lymph flowing through the lymphatic vessel. An ample supply of oxygen will be required to maintain rhythmic contractions, which act as a driving force for the propulsion of lymph (4). The amount of oxygen in lymph and the rate of flow in lymphatics are extremely low compared with those in arterial blood and in arteries (5). Hence, it may be comprehensible from the teleological point of view that the presence of blood capillaries within the well-developed smooth muscle layers is essential for bovine mesenteric lymphatics to maintain vigorous spontaneous activity. We have also found blood capillaries within the smooth muscle layers of the canine ureter and portal vein which were known to have spontaneous rhythmic contractility. Blood capillary has been rarely found in the media of bovine mesenteric arteries.

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