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# Electrical Activity of Lymphatic Smooth Muscles (39787)

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It has been noted that rhythmical contractions occurred in the lacteals of rats and guinea pigs and that the rate of these contractions increased by local application of adrenaline and by stimulation of the splanchnic nerves (1). Similar spontaneous contractions were also found in the popliteal lymphatics of rats, mice, and guinea pigs (2) and in the bovine mesenteric lymphatics (3). There have been only two previous reports of electrical activity of lymphatic smooth muscle (4, 5). The present experiments were designed to study spontaneous electrical activity in bovine mesenteric lymphatics by means of the sucrose gap technique.

*Materials and methods.* Segments of mesenteric lymphatics, between 2 and 5 mm in outer diameter, were dissected from the fresh mesenterics of recently slaughtered cattle. Spiral strips, about 20 mm long and 1 mm wide, were cut from these segments and kept in a standard bathing solution of the following composition in mmole/liter:NaCl 154.0, KCl 5.6, $CaCl_2$, 2.2, $NaHCO_3$, 8.0, glucose, 5.5. The solution was kept at 37° and continuously aerated with 100% $O_2$ to give a pH of 7.4. 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 standard solution or with one of the test solutions containing noradrenaline, acetylcholine, or manganese. Another end was anchored at a fixed point and depolarized by $K_2SO_4$ Locke's solution, which had the composition (mmole/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 displayed on a dual beam synchroscope (Iwatsu DS-5015) and recorded by a direct writing oscillograph (Sanei Sokki 8S). The strips were maintained at a tension of 100–250 dynes and a total of 11 preparations were examined. Seven of these showed pronounced spontaneous activity. Resting potentials were measured by replacing the standard solution with $K_2SO_4$ Locke's solution (6, 7).

*Results and discussion.* Figure 1 demonstrates membrane action potentials 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 (about 100 msec in duration), and final slow repolarization. The average value of resting potential in lymphatic smooth muscles was estimated to be 32.7 ± 4.2 mV, which was a little less than that in vascular smooth muscles obtained by intracellular electrodes (8, 9).

As shown in Fig. 2, a slight but long-lasting depolarization superimposed by frequent discharges of action potentials took place in quiescent strips immediately after application of noradrenaline. These electrical changes were followed by a gradual rise in smooth muscle tone and the appearance of the phasic contractions which had one-to-one correspondence to the action potentials.

The administration of acetylcholine in a relatively high concentration to quiescent





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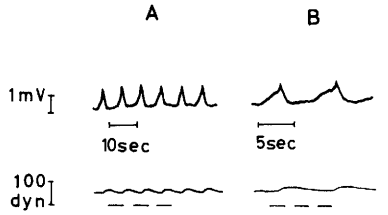


FIG. 1. Spontaneous electrical and mechanical activities of bovine mesenteric lymphatics recorded by the sucrose gap method. Upper and lower tracings are action potentials and isometric phasic contractions, respectively. The action potentials have one-to-one correspondence to the contraction waves. In (B) the time scale is $2^2/_3$ times as long as that in (A). Broken lines indicate zero tension level.

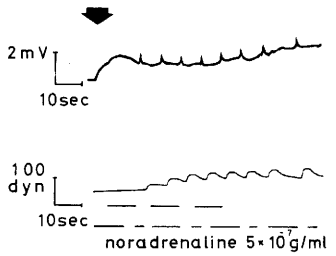


FIG. 2. Effect of noradrenaline on quiescent lymphatic smooth muscles. Upper and lower tracings are electrical and mechanical changes produced by $5 \times 10^{-7}$ g/ml of nordrenaline applied at the moment indicated by the arrowhead. A long-lasting depolarization is superimposed by frequent discharges of action potentials. These electrical changes are followed by a gradual rise in smooth muscle tone and the appearance of phasic contractions. Broken and dashed-dot lines indicate zero tension level and the period of noradrenaline administration, respectively.

strips induced a transient depolarization followed by the occurrence of action potentials (Fig. 3). Each action potential of the pacemaker type preceded a contraction wave. This effect, observed in four of nine cases, is inconsistent with the observation by Mawhinney and Roddie (3) that acetylcholine ($10^{-9}$–$10^{-7}$ g/ml) had little effect on the rate and amplitude of spontaneous contractions of bovine mesenteric lymphatics. The discrepancy may not be fully explained by the high concentration of acetylcholine we used, since other authors (4, 5) have shown that acetylcholine in a concentration of $10^{-7}$ g/ml had a positive chronotropic effect on the valved segments of lymphatics in guinea pigs and rats. The finding obtained by the present authors that the specimens which contained no valvular region were sensitive only to noradrenaline but not to acetylcholine may suggest the possibility that acetylcholine-sensitive smooth muscles might locate only in the valvular regions of the lymphatic vessels, though Mawhinney and Roddie made no mention of whether or not their specimens had contained the valvular region. Some differences in ultrastructure were recognized between smooth muscles in the valvular region and those in the central region. The electron microscopic findings will be reported elsewhere.

So far as we are aware, no investigation has been carried out on the electrical activity of lymphatic smooth muscles, with the exception of the following two. In 1966, Mislin (5) succeeded for the first time in recording extracellular action potentials of the mesenteric lymphatics in guinea pigs by using suction electrodes. He made no mention of the level of membrane resting potential and of the shape of action potential. Recently, Orlov *et al.* (4) published a paper on contractile and electrical activities of lymphatic smooth muscles in rats. The value of the membrane resting potential obtained by the sucrose gap method was quite similar to ours. They demonstrated only one simultaneous recording of membrane action potentials and isometric contraction waves by the same method. No detailed description was given about the configuration of action potentials. Each one of these action poten-

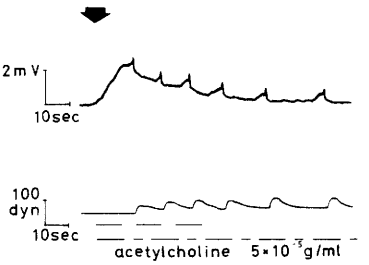


FIG. 3. Effect of acetylcholine on quiescent lymphatic smooth muscles. Upper and lower tracings are electrical and mechanical changes produced by $5 \times 10^{-5}$ g/ml of acetylcholine applied at the moment indicated by the arrowhead. A transient depolarization is followed by the occurrence of action potentials. Each action potential is accompanied with a contraction wave. Broken and dashed-dot lines indicate zero tension level and the period of acetylcholine administration, respectively.

tials was shown to appear at the moment at which the isometric tension reached a maximum. In other words, the contractile activity made its appearance about 2 sec after completion of the preceding electrical activity. Such a time lag between electrical and contractile activities seems so unusual that there is some doubt as to whether or not the electrical changes they recorded were true action potentials.

The ionic mechanism of lymphatic electrical activity was also conjectured by using the appearance of contraction waves as an index of discharge of action potentials. In our preparations, as shown in Figs. 1–3, every one of the action potentials was always accompanied by a phasic contraction, and vice versa. Hence, we could estimate whether or not the action potentials were discharged from the mechanogram alone.

The rhythmicity and amplitude of spontaneous contractions were not affected by tetrodotoxin, which is known to be a selective blocking agent for sodium rapid carrier mechanism (Fig. 4A). As a matter of course, this does not directly rule out the possibility that sodium ions participate in the excitation of the lymphatic smooth muscle, since the sodium-based action potentials of some smooth muscles have been shown not to be blocked by tetrodotoxin (10). Application of manganese abolished the spontaneous contraction within 2 min (Fig. 4B). Simultaneous recordings of electrical and mechanical activities proved the concurrent disappearance of action potentials (Fig. 4D). As is well known, manganese selectively blocks calcium influx through excitable cell membrane. Direct electrical stimulation could evoke contractile responses of the specimens under the influence of $10^{-6}$ g/ml of manganese. The phasic contractions immediately disappeared in a calcium-free environment. The contractions made their appearance soon after one addition of barium chloride (Fig. 4C). These results suggest that calcium current probably plays a determining role in producing spike discharge in lymphatic smooth muscles, as has often been the case with other smooth muscles.

*Summary.* Membrane action potentials of bovine mesenteric lymphatics were recorded simultaneously with isometric contractions by the use of the sucrose gap method. The action potentials, about 3 sec in duration, always had one-to-one correspondence to the contraction waves. Each action potential consisted of three phases, i.e., initial slow depolarization, spike, and slow repolarization. The application of noradrenalin elicited a slight but long-lasting depolarization superimposed by frequent discharges of action potentials. The administration of acetylcholine in a relatively high concentration to the preparations induced a transient depolarization followed by the occurrence of action potentials. From the effects of tetrodotoxin, manganese, calcium-

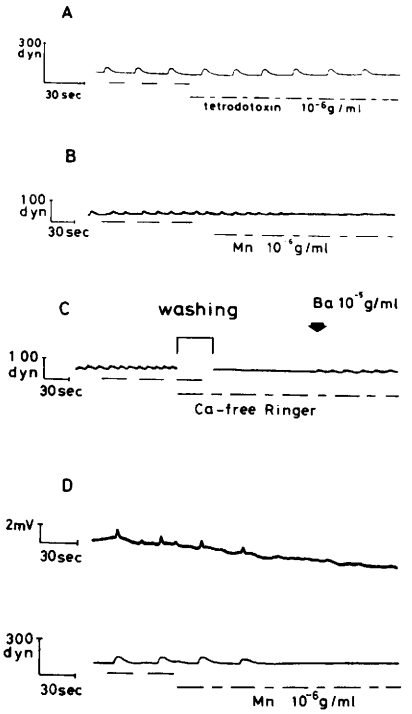


FIG. 4. Effect of tetrodotoxin, manganese, and calcium-free environment on spontaneous contractions of lympatics. Spontaneous contractions are not affected by tetrodotoxin (A) but are abolished by manganese (B). Spontaneous contractions, which have been suppressed in a calcium-free environment, reappear immediately after addition of barium chloride (C). Action potentials disappeared simultaneously with contraction waves under the influence of manganese (D). Broken lines indicate zero tension level. Dashed-dot lines denote the period of drug administration in (A), (B), and (D) and that of immersion in a calcium-free Ringer solution in (C). An arrowhead indicates the moment of $BaCl_2$ administration.

free environment, and barium chloride on the spontaneous contractions, it was suggested that calcium current may probably play a major role in producing spike discharge in bovine lymphatic smooth muscles.

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