PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 350-352 (1978)

Transmembrane Potentials in Bovine Lymphatic Smooth Muscle (40346)

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The use of intracellular electrodes for smooth muscle was introduced by Bülbring and Hooton (1), and this method of recording has since been applied to a variety of smooth muscles. Specifically, intracellular studies of the electrical activity of vascular smooth muscle in the frog have been reported by Funaki (2), in turtle arteries and veins by Roddie (3), and in rat and guinea pig small mesenteric arteries and veins by Trail (4), Speden (5), Nakajima and Horn (6), and Ito and Kuriyama (7). Recently, by means of the sucrose gap method, the present authors have successfully recorded membrane action potentials of bovine mesenteric lymphatics simultaneously with phasic contraction waves which had one-to-one correspondence to the action potentials, and the authors suggested that calcium current may probably play a major role in producing spike discharge in bovine lymphatic smooth muscles (8). The lymphatics exhibited, even *in vitro*, vigorous spontaneous contractile activity. Contractions of the lymphatic smooth muscles were also induced by 5-hydroxytryptamine (5-HT), prostaglandin $F_2\alpha$, noradrenaline, histamine, dopamine and acetylcholine. The smooth muscles were particularly sensitive to 5-HT (9). In the following experiments we have studied the membrane activity of single cells of bovine mesenteric lymphatics with intracellular microelectrodes.

Materials and methods. Segments of mesenteric lymphatics, between 0.5 and 3 mm in outer diameter, were dissected from the fresh mesenterics of recently slaughtered cattle. Longitudinal strips, about 5 mm long and 1 mm wide, were cut from these segments and kept in a chamber containing a modified Locke's solution of the following composition in mmoles/liter: NaCl 154.0, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 1.8, glucose 5.5. The solution was maintained at 37° and continuously bubbled with 100% O_2. It was revealed by repetitive direct measurements with a pH meter (F3, HORIBA) that the solution was kept at an approximately constant pH of 7.4 for more than 6 hr. The strip was mounted with the outer surface upward on a thin silicon rubber plate consisting of the bottom of the chamber. Connective and adipose tissues covering the outermost longitudinal smooth muscle layer were gently removed. A microelectrode filled with 3 M potassium chloride, with tip resistances of about 50–80 MΩ and diameter less than 0.5 μm, was inserted into the wall of the specimen with a micromanipulator under the binocular microscope with incident illumination. A nonpolarizing Ag–AgCl wire was used as a reference electrode. These two electrodes were connected to a high input resistance preamplifier (Nihon Koden MEZ-8101), the output from which was displayed on a dual beam synchroscope (Iwatsu DS-5015) and recorded by a data recorder (TEAC R-351F).

Results and discussion. Spontaneous contractile activity was observed with most of the lymphatic strips under the binocular microscope when incubated in the warm modified Locke's solution. The rhythm of the contractions was regular and highly sensitive to environmental temperature. The beating rate was 4–6/min at 37°. It was almost doubled by the elevation of temperature up to 40°, keeping the specimen length unchanged. Figure 1 shows typical patterns of spontaneous electrical activity in lymphatic smooth muscle. A burst of spike discharges was frequently observed in association with a contraction wave and lasted for several seconds or longer. In this record the resting potential measured at maximum polarization between one spike and another was about −50 mV. The average value of the resting membrane potential was -49 ± 2.4 mV in 10 experiments. The resting potential sometimes showed slight rhythmic fluctuations or slow waves at various intervals, rarely with an after-hyperpolarization which resembled that in visceral smooth mus-

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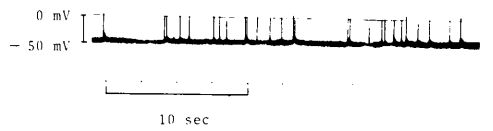

FIG. 1. Spontaneous electrical activity in lymphatic smooth muscle.

cles (5). The resting potentials seemed lower in the lymphatic smooth muscle with spontaneous activity than in that without the activity. The firing of lymphatic action potentials could be classified into two patterns, i.e. (1) short trains consisting of several spikes, and (2) single spikes or irregular spike discharges. The amplitude of the action potentials ranged from 39 to 57 mV (mean 47 ± 4.6 mV). Occasionally the action potentials showed a slight overshoot of less than 5–7 mV. In some cases, as shown in Fig. 2, many of the action potentials were superimposed upon the rising phase of the slow fluctuations. The amplitude of the slow waves was about 10 mV and was considerably smaller than that in visceral smooth muscle cells (10). The duration of the fluctuations was about 400–1200 msec. Frequently, the discharge of spike appeared to be triggered by the slow depolarization. Figure 3 represents a typical action potential of lymphatic smooth muscles. The configuration of the action potential is similar to that of smooth muscles in the *taenia coli* (11) or portal vein (7) of the guinea pig. The action potential usually consisted of three phases, i.e. rapid depolarization, fast, and slow repolarization. The duration of the action potentials was about 40–50 msec at 37° but prolonged with lowering environmental temperature down to 35°. The prolongation was usually attributed to the extension of the repolarization phase of the action potentials. In the previous paper (8), it was reported that the rhythmicity and amplitude of spontaneous contractions in bovine mesenteric lymphatics were not affected by tetrodotoxin, which is known to be a selective blocking agent for sodium rapid carrier mechanism. In the present experiments, it was also recognized that the level of resting potentials and the configuration of action potentials were not affected by tetrodotoxin (Sigma).

The average of resting membrane potentials in lymphatic smooth muscles were lower than those in visceral smooth muscles (12)

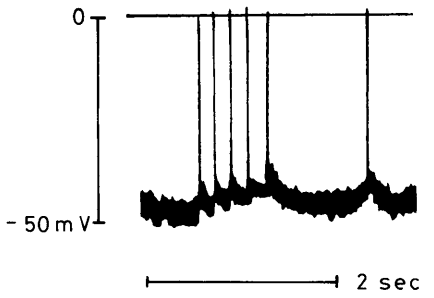

FIG. 2. Lymphatic action potentials superimposed upon the rising phase of slow fluctuations in the resting potential.

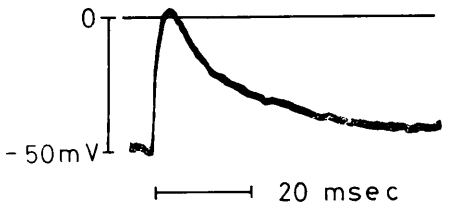

FIG. 3. A typical action potential of lymphatic smooth muscle.

but somewhat higher than those in vascular smooth muscles (4, 5). The lymphatic smooth muscle cell is so small that it may easily be damaged during impalement with a microelectrode. It should be noted, however, that when successful impalement was maintained over a long period of time no change was observed in the value of the resting potential. In the taenia coli, Bülbring (13) noticed that the extension of the smooth muscle, within certain limits, led to an increased tension accompanied by an increased oxygen consumption, and the membrane potential was found to depend upon the degree of stretch. It might be possible that the level of transmembrane potentials could also be influenced by the degree of stretch in lymphatic preparations. In the previous paper (8) it was reported that by means of the sucrose gap technique the average value of resting potentials in lymphatic smooth muscles was estimated to be 32.7 ± 4.2 mV. In the sucrose gap technique, in principle, there are the following controversial points in regard to the estimation of transmembrane potentials: (1) short-circuiting between the electrodes exists in appreciable quantities and (2) the sucrose solution fails to replace all the ions lying in the interstitial spaces. Artifacts due to junction potentials should not be overlooked in

sucrose gap experiments. These may offer an explanation for the differences between the values of resting potentials in lymphatic smooth muscles recorded by the intracellular microelectrode and those by the sucrose gap technique. As represented in Fig. 2, the lymphatic action potentials were frequently superimposed upon the slow fluctuations. When the depolarization due to the fluctuations reaches a critical level, the firing of a single action potential or a burst of spikes may take place. Spontaneous subthreshold fluctuations in membrane potential have been recorded in various muscular and nervous tissues of both vertebrate and invertebrate animals (14, 15). These fluctuations are generally considered to be the basis of rhythmical firing of action potentials and hence of spontaneous mechanical activity. In smooth muscle, subthreshold activity appears to be at least of two kinds. In some cases it is nearly sinusoidal in appearance and is referred to as "slow waves". In other cases the membrane potential depolarizes slowly to a point where threshold is reached and an action potential is initiated. The configurations of the slow fluctuations in lymphatic smooth muscles are similar to those in the rabbit colon (16) or in the guinea pig jejunum (17). It has been reported that spontaneous contractions of visceral smooth muscles are caused by repetitive firing of action potentials. Each burst of spike discharges in lymphatic smooth muscle is well coordinated with the mechanical event amounting to a spontaneous contraction wave under the binocular microscope. The amplitude and duration (47.8 ± 9.4 msec) of action potentials in lymphatic smooth muscles were lower than those in visceral smooth muscles (12) but somewhat higher than in vascular smooth muscle (4, 5). By use of the sucrose gap technique, the present authors (8) reported that the lymphatic action potentials were similar in appearance to the pacemaker potentials recorded from some other smooth muscles. As a matter of course, the recordings by the sucrose gap technique are extracellular ones and represent compound potentials of a lot of cells present in the preparations. In the present experiments, on the other hand, the transmembrane activities of lymphatic smooth muscles were recorded from the effector cells located in the margin of the preparations in order to avoid the influence of vigorous spontaneous contractions. The activities were not recorded from the pacemaker sites. This may explain the difference in the configurations of lymphatic action potentials in the present and previous reports.

Summary. Transmembrane potentials in smooth muscle fiber of bovine mesenteric lymphatics have been studied with the aid of an intracellular microelectrode technique. Resting potentials ranged from −41 to −57 mV. In most of the preparations, the slow fluctuations in the resting potentials were recognized, amplitude and duration of which were about 10 mV and 400–1200 msec, respectively. A burst of action potentials was associated with a spontaneous contraction wave. The amplitude of the action potentials ranged from 39 to 57 mV. The duration of the action potentials was 47.8 ± 9.4 msec in 10 experiments. The magnitude of occasional overshoot was a few millivolts. The level of the resting potentials and the configuration of the action potentials were not affected by tetrodotoxin.

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Received May 12, 1978. P.S.E.B.M. 1978, Vol. 159.