Membrane Potentials of Smooth Muscle Cells of Isolated Resistance Vessels[1] (37625)

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Since Funaki's (1, 2) first membrane potential recordings from arterioles in the frog, there have been few reports of microelectrode studies of resistance vessels; those carried out on mesenteric vessels in intact rats and guinea pigs (3–5) showed spontaneous spike discharges, those done on isolated ear and mesenteric arteries of the rabbit (6) did not. Microelectrode study of vascular smooth muscle from other sources indicates that the portal vein has spontaneous action potentials and that this activity is augmented when the cells are stimulated with epinephrine (7). On the other hand, the pulmonary artery has no spontaneous membrane potential activity and no action potentials have been observed during its contractile response to norepinephrine (8).

The present microelectrode study was carried out on the small resistance arteries that regulate blood pressure and flow distribution. The study had two objectives: (i) to see if there are differences in electrical properties of the membrane associated with two different types of smooth muscle, that of the skeletal muscle artery that does, and that of the mesenteric artery that does not, develop spontaneous tone in the isolated bath (9), and (ii) to determine if the constriction of these arteries in response to KCl or epinephrine stimulation is accompanied by action potentials or by a change in membrane potential.

Methods. Vessels studied were from both rats and guinea pigs; they were branches of the superior mesenteric artery and of the saphenous and the deep caudal epigastric arteries supplying skeletal muscle. A segment of a small artery (100–300 μm o.d.) was removed from an animal and attached to a small plexiglass disk by fixing both overhanging ends of the vessel segment by a thread tied around the edge of the disk. The disk carrying the vessel was then mounted in a muscle bath (volume ca. 30 ml) which was continuously perfused with physiological salt solution (PSS) at 37°. The composition of the PSS was (m*M*): NaCl, 119.0; KCl, 4.7; $MgSO_4$, 1.17; $CaCl_2$, 1.6; KH_2PO_4, 1.18; dextrose, 5.5; sucrose, 50.0; and calcium versenate, 0.026. Membrane potentials were recorded by intracellular microelectrodes; a detailed description of the method is given in a previous paper (10). Records were obtained by an ink-writing oscillograph (Grass polygraph, response flat to 40 cps). Stimulating agents (epinephrine, KCl) were added by injection directly into the bath. Since the solution in the bath was constantly agitated by the bubbles of the aerating gas mixture (95% O_2, 5% CO_2), quick mixing of the added agents occurred. The effectiveness of the equipment was established in preliminary experiments with the portal vein of rats and guinea pigs. Here continuous records of spontaneous spike activity from a single cell could be obtained relatively easily, for an hour or more.

Results. With the small vessels, no measurements of changes in tension were made during the intracellular studies but mechanical records were made in separate experiments. For this purpose the vessel segment was mounted as described above and, additionally, fixed over its entire length by a fine

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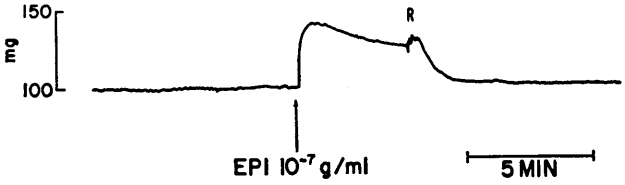

FIG. 1. Effect of epinephrine on tension of an isolated resistance vessel. At the arrow, epinephrine (to give a concentration of 10^{-7} g/ml in the bath) was added. The slow drop in tension after the initial peak was caused by a continuous decrease in the epinephrine concentration as the bath was perfused by PSS during and after the addition of the drug. At R the bath was rinsed with normal PSS (rat, skeletal muscle vessel, ca. 200 μm o.d.).

(50 μm) tungsten wire threaded through its lumen. A second wire was hooked through the vessel wall so that a small area of the wall containing circularly-running muscle fibers could be raised up and connected to a force-displacement transducer. The force stretching the wall was adjusted to approximately 100 dynes. Under these conditions reproducible contractions were obtained in response to epinephrine (Fig. 1) or KCl in concentrations subsequently employed in the microelectrode studies. Except for some very slow spontaneous fluctuations in tension (period duration 5–10 min) which were occasionally observed in the skeletal muscle vessels, no indication of any spontaneous mechanical activity was present.

In the microelectrode studies the membrane potential was found to be stable (Fig. 2A), ranging between 42–58 mV (mean 48 mV, SD $\pm$ 5 mV, $n = 36$). In spite of the different origins of the vessels, there were no significant differences (tested by analysis of variance, computed F ratio $= 0.21$; $F_{.95} = 2.90$) between the values obtained from the several groups. There was no suggestion of spontaneous spike discharge.

Increases in the KCl concentration of the PSS induced a slow, graded depolarization (an increase from 4.7–50 mM caused a drop in membrane potential of about 35 mV) which was reversed after the KCl concentration was returned to normal (Fig. 2B and C). No spike discharge was evoked by stepwise membrane depolarization by KCl. Epinephrine in increasing concentrations (10^{-8}–10^{-6} g/ml) also led to a graded depolarization (Fig. 3). The maximum drop in potential was approximately 20 mV at high epinephrine concentrations. At the plateau of a maximum epinephrine depolarization an increase in the KCl content (to 50 mM) caused an additional decrease in membrane potential (Fig. 3, lower tracing). Epinephrine also failed to elicit any spike activity.

Discussion. The absence of spontaneous spikes in these experiments is in contrast to the findings of spike discharge in *in vivo* experiments with small mammalian arterial vessels (3–5) but in accordance with the results so far obtained from isolated small arteries (6). Steedman (5) assumed that the spontaneous spike activity which she observed in resistance vessels of intact animals might be evoked by action of the autonomic nervous

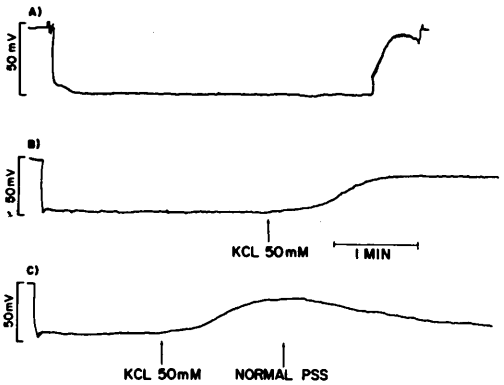

FIG. 2. Membrane potential of isolated resistance vessels recorded from single cells. (A) Stable membrane potential after insertion of the microelectrode and spontaneous dislocation of the electrode after ca. 4 min. (Rat, skeletal muscle vessel, ca. 200 μm o.d.). (B) Depolarization caused by an increase in the KCl concentration of the PSS (from 4.7 to 50 mM). Drop of membrane potential, ca. 35 mV. (Guinea pig, mesenteric vessel, ca. 150 μm o.d.). (C) KCl depolarization and subsequent repolarization in normal PSS (guinea pig, skeletal muscle vessel, ca. 200 μm o.d.).

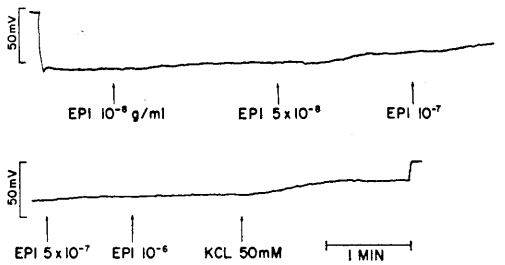

FIG. 3. Effect of epinephrine on membrane potential of an isolated resistance vessel (continuous recording). Epinephrine in increasing concentrations (10^{-8} to 10^{-6} g/ml) causes graded depolarization, with a maximum of approximately 20 mV. KCl (50 m*M*) induces additional depolarization. Spontaneous dislocation of the electrode at the end of the lower tracing (guinea pig, skeletal muscle vessel, ca. 200 μm o.d.).

system. However, other factors may play a role in facilitating spike discharge *in situ*; *e.g.*, the tension in the vessel wall caused by intraluminal pressure, the continual mechanical stimulation of the vessel wall by the pulsatile pressure and humoral factors in the blood. Speden (4, 6) found a difference between membrane potential values of isolated small arteries and those of intact animals, and he believed that this difference was responsible for the difference in their electrical behavior. This cannot be a full explanation for the absence of spikes in isolated vessels in the current study. If this were the case, it would be expected that the graded depolarization caused by KCl or epinephrine would, at a certain point, reach the threshold for action potentials. The lack of spike activity even in vessels that exhibit "myogenic tone" suggests that the mechanism for this tone differs from that of other types of smooth muscle (*e.g.*, intestinal smooth muscle, portal vein, etc.) where spontaneous tension development is usually associated with phasic electrical phenomena of the cell membrane. The tonic contraction of smooth muscle of arteries isolated from skeletal muscle may be due to a graded contraction of nonelectrical origin.

It is clear that two distinct activation pathways exist. In one pathway, exemplified by that responsible for rhythmic contractions of the portal vein, there is a causal relationship between the phasic electrical phenomena of the plasma membrane and the resultant mechanical response. Normally the mechanical event of the portal vein occurs only in association with action potentials of this smooth muscle; variations in the pattern of action potentials result in altered mechanical responses (11). This is electrical activation. Coupling between the electrical phenomena of the membrane and the mechanical response of this muscle requires calcium. In a calcium-free solution, the addition of norepinephrine may initiate action potentials in the portal vein without a contractile response (12). A quite different pathway must be responsible for the contraction that occurs in the absence of a change in membrane potential. This is clearly demonstrated when smooth muscle in a depolarizing potassium solution contracts in response to epinephrine or to other agonists (13, 14). This is nonelectrical activation. The mechanical response resulting from this type of activation is also calcium dependent (15).

In the current study, activation of smooth muscle by epinephrine was not accompanied by action potentials or any phasic activity of the membrane potential. There was, however, a slowly developing depolarization. This graded depolarization and absence of action potentials in response to epinephrine in the small arteries is similar to that reported for larger arteries (8, 13, 16–18). It appears that alpha adrenergic activity in arterial smooth muscle is commonly accompanied by relatively small, graded changes in potential. The question arises whether these slight depolarizations are responsible for the mechanical response, or whether they are merely epiphenomena caused by a change in membrane conductance (13, 18), that accompanies, but does not cause, the increased concentration of activator calcium responsible for contraction. Mekata and Niu (18) found that small doses of epinephrine induce contraction in isolated common carotid artery, without change in membrane potential, whereas higher concentrations cause both contraction and depolarization. Similarly, in the current study the change in membrane potential in response to low concentrations of epinephrine was remarkably small, whereas the same low concentrations of epinephrine cause distinct con-

tractions. Furthermore, Haeusler (8) has observed that verapamil eliminated the contraction of vascular smooth muscle produced by norepinephrine without altering its membrane-depolarizing effect. In none of these responses to alpha adrenergic activation has an action potential been observed. It seems unlikely that the electrical change in the isolated small arteries in response to epinephrine is related to the mechanical event; apparently in this polarized smooth muscle the contractile response to epinephrine results from noneleotrical activation.

Summary. The microelectrode technique was used to measure membrane potentials from branches of the saphenous and deep caudal epigastric arteries supplying skeletal muscle and of the superior mesenteric artery of rats and guinea pigs. We found no significant differences in membrane potentials of cells from the different sources. Although KCl and epinephrine produced some depolarization of this vascular smooth muscle, the magnitude of the depolarization did not appear to reflect the magnitude of the contractile response and there was no suggestion of a spike discharge; activation of the contraction produced by these agents must be initiated through a nonelectrical pathway.

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