Electrical Quiescence of Pulmonary Artery Smooth Muscle During Sympathomimetic Stimulation

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Some recent reviewers\(^1\) of the electrophysiology of smooth muscle have suggested that spike activity is normally a necessary prelude to excitation of the contractile mechanism. This conclusion has been based mainly on studies of tissues other than vascular muscle. Consistent with this view is the demonstration, by intracellular microelectrode technique, of induced or spontaneous muscle action potentials in the small blood vessels of the frog\(^3\) and rat\(^4\) and those associated with contraction in the turtle aorta and vena cava.\(^5\) However, investigations involving extracellular recording have failed to show action potentials in some larger mammalian blood vessels under resting conditions\(^6\) or following stimulation with epinephrine.\(^8\) Intracellular technique has been applied to a vessel of the latter type, the rabbit mesenteric artery, only to measure the magnitude of the resting membrane potential.\(^9\) We designed the present experiments in order to measure changes in membrane potential associated with contraction of the smooth muscle of a large mammalian blood vessel thereby providing more precise information regarding excitation-contraction coupling.

The present work was carried out on the sympathetic nerve-pulmonary artery preparation of the rabbit.\(^10\) In this preparation the membrane potential of the muscle cell may be recorded during a contraction in response to a chemical agent or to stimulation of the sympathetic nerve supply of the blood vessel. Preliminary results of a portion of this work have been reported previously.\(^11\)

Methods

The distal 7 to 10 mm of the rabbit pulmonary artery with the right recurrent cardiac nerve were rapidly removed after the animal had been stunned and bled.\(^10\) Rabbits weighing between 3.0 and 3.5 kg were used. The artery ring was turned inside out and mounted almost horizontally between a rigid plastic support and a platinum wire loop connected to a force-displacement transducer. The tissue was so arranged that the nerve entered the upper layer of the stretched arterial loop halfway between the two supports. It was completely immersed in 20 ml Krebs solution which was kept at 38°C and equilibrated with 95% O\(_2\) and 5% CO\(_2\) in a jacketed tissue bath. For the purpose of indirect stimulation the nerve was pulled through two immersed, uninsulated platinum ring electrodes placed approximately 5 mm from the artery. It has previously been observed that contraction produced by this mode of stimulation is solely neurogenic.\(^10\) A resting tension of 10 g was employed in order to minimize movement of the tissue. The stress in the arterial wall produced by this tension is of similar magnitude to that which occurs during systole in the intact animal.\(^12\)

Conventional KCl-filled rigidly mounted glass micropipettes of 20 to 50 megohms were used to impale single muscle cells usually in the region subjacent to the site of entry of the nerve. The pipettes were connected to a cathode follower-amplifier (Medister A-35) and the potential changes monitored on a cathode ray oscilloscope. The grid current was less than 10\(^{-10}\) amp and the input time constant of the cathode follower probe with a 48 megohm electrode in place was 27 \(\mu\)sec. Both the potential changes and the isometric tension changes were simultaneously recorded on a Grass polygraph.

The arterial ring was stimulated indirectly through the nerve using a Grass stimulator and Argonaut isolation transformer (LIT089), mostly by trains of stimuli of supramaximal voltage (2 msec duration). Since the contractile response...
and membrane potential change following potassium chloride addition were the same whether or not compensatory reductions in sodium chloride were made under these experimental conditions, potassium chloride was added directly in small volume to the tissue bath. Concentrations of l-norepinephrine bitartrate are in terms of base.

**Results**

**RESTING MEMBRANE POTENTIAL**

Since the adventitia contains an abundance of connective tissue, the microelectrode was inserted into the artery from its intimal surface, and, in most experiments, only passed through three or four layers of smooth muscle cells. As the electrode slowly advanced, a series of sudden well-defined potential changes was seen, presumably associated with penetration of successive cell layers. Between these increases the potential difference abruptly returned to zero. Preliminary experiments showed that the mean resting potential of the subintimal muscle cells did not differ from that of deeper cells, nor did it vary in different portions of the same layer in the artery. The average resting potential of the cells of a given tissue remained unchanged under the conditions of the experiment for six hours or more. The resting potential was independent of the equilibrium resting tension up to strains of 10 to 15 g. Strains greater than this were associated with a reduction in potential.

**FIGURE 1**

Distribution of values for resting membrane potential of pulmonary artery smooth muscle.

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The effect of sudden changes in muscle length on the membrane potential was not examined.

The distribution of the resting membrane potentials recorded in preparations from 53 rabbits is shown in figure 1. The median potential is 51.5 mv; the mean 51.5 ± se 0.34 mv (number of observations = 517). A sudden, well-defined and sustained downward deflection (negative) of the oscilloscope trace, with little change in electrode resistance and base line, were the main guiding signs for a successful impalement.

**EFFECT OF SYMPATHETIC NERVE STIMULATION**

An isometric tension increase of between 0.5 and 1.5 g followed repetitive stimulation of the recurrent cardiac nerve for ten seconds. The contraction started about two seconds after the beginning of indirect stimulation, had a half time of seven seconds, and continued to develop several seconds after the termination of the stimulation period.

In 12 experiments, when the electrode remained within a cell throughout contraction, no change in membrane potential during nerve stimulation or contraction was recorded (fig. 2). In several other experiments, either slight depolarization (1 to 3 mv) or even hyperpolarization of several mv was found which lasted an unpredictable length of time; the
FIGURE 3

Effect of sympathetic nerve stimulation (N.S., 2 msec duration) on membrane potential and muscle tension. A and B: Amplified potential, tension, and time scales were used. Stimulation at 1, 5, and 25 cycles/sec and 120 v produced downward deflections as artifacts. C: Oscillographic tracing of membrane potential. Resting potential was 48 mv. At beginning of tracing (bottom), no spontaneous electrical activity is seen. (Short vertical lines are due to graticule time divisions.) In the middle of fourth sweep a single shock artifact is registered, followed by tetanic stimulation with no resultant spikes or slow waves. At the end of stimulation, muscle contraction amounted to dislodge the microelectrode.
Effect of l-norepinephrine (NEP) on membrane potential and muscle tension.

Membrane potential changes and contractile response were recorded following the addition of l-norepinephrine (0.01 and 0.1 μg/ml) to the tissue bath. On a number of occasions during which the microelectrode tip was maintained within the cell up to 90 seconds after the beginning of contraction, no action potentials or other changes in the membrane potential were observed (fig. 4). In other experiments, a random group of cells was impaled before and after exposure to l-norepinephrine (fig. 5). There was no difference in the resting potential before and after the addition of submaximal doses of the drug, nor was any action potential recorded.

EFFECT OF POTASSIUM

When the potassium chloride in the bathing solution was increased to four times its normal concentration (5.9 mM), the membrane potential began to decrease prior to the onset of contraction. A plateau of depolarization was attained well before contraction was complete (fig. 6). The time course of these changes was followed in six experiments by impaling muscle fibers before and after in-
creasing the concentration of potassium chloride, and by keeping the microelectrode tip within muscle cells during the early part of the contractile response. Depolarization commenced within 15 seconds of the potassium increase, had a half time of 0.9 minute, and was completed within 3 minutes. The mean decrease was 20 mv. In comparison, the latent period between potassium addition and contraction was 0.8 minute, the half time of contraction, 3.1 minutes. The maximal tension increase of approximately 6 g was attained after 12 minutes (fig. 7). This process was reversible and reproducible. A few experiments conducted with equivalent amounts of potassium sulfate and potassium methylsulfate suggested that depolarization with these salts was more complete than with the chloride.

Discussion
The unexpected finding of this investigation is the electrical quiescence of pulmonary artery vascular muscle at rest and during contraction induced by sympathetic nerve and amine stimulation. This was an invariable phenomenon even though, under the same experimental conditions, electrical activity was easily recorded in other smooth muscle tissues.

Since electrical quiescence is infrequent in smooth muscle, the possibility was entertained that the membrane potentials being recorded from the artery might not arise from contractile smooth muscle cells. This possibility, however, can be discounted by the following considerations. Electron microscopic studies demonstrated that the arterial intima is composed of a thin monolayer of endothelial cells and it has been stated that the only cellular constituents of the media of an elastic vessel are smooth muscle cells. In the present experiments, the endothelial cells could only be responsible for the initial potential changes while the electrode was advanced into the artery wall. However, these changes were routinely disregarded and used solely to indicate when the tip had reached the wall surface. Nerve fibers in the pulmonary artery appear too fine to be speared. The extracellular elastic and collagenous elements are also unlikely to be significant, as the potential level returned to the base line between well-defined negativities which responded to potassium salt additions.

It is possible that not all muscle fibers in this preparation are supplied by sympathetic neurones in the recurrent cardiac nerve, but unlikely that these would have been con-
Membrane Potential of Arterial Muscle

Persistently selected by the microelectrode. The maximal contractile response to nerve stimulation is not very much smaller (30 to 50%) than that to I-norepinephrine – an observation that questions extreme paucity of motor innervation. In addition, it has been stated that autonomic nerve fibers probably exert a diffuse influence on smooth muscle cells. A wide variety of excitability may exist among the individual cells, as shown in the urinary bladder, and might thus diminish the chance of detecting the nerve stimulation effect. Nevertheless, if the presumable transmitter I-norepinephrine is to cause a junction potential after the nerve stimulation, as in the guinea pig vas deferens, large amounts of this drug would be expected to cause a detectable depolarization in most, if not all, muscle cells. No significant change was ever observed. Therefore, it is concluded that electrical quiescence during sympathetic nerve and amine stimulation is a characteristic of this tissue.

The magnitude of resting muscle membrane potential of the rabbit pulmonary artery, as well as the mesenteric artery, is comparable to that of the well-investigated guinea pig taenia coli, although the depolarizing effect of potassium chloride (four times normal concentration) appears smaller in the latter. Otherwise, these present results contrast with the findings in most smooth muscle of other origins. A few vascular preparations subjected to intracellular recording displayed either induced action potentials, spontaneous discharges, or slow waves. These activities may be related to the proposed interfiber conduction, which is absent in some blood vessels perhaps including the pulmonary artery. The smooth muscle cells of the rat aorta, another elastic artery, have been described as having a rudimentary structure.

Some extravascular smooth muscle also contracts in response to adrenergic stimulation. That of guinea pig vas deferens manifests spontaneous small potentials, junction potentials, and spike discharges which were attributed to releases of norepinephrine, although indications have since accumulated that its sympathetic innervation, the hypogastric, contains complex components. 

The contraction of guinea pig esophagus smooth muscle elicited by epinephrine is accompanied by depolarization and initiation of spike activity. These results have led to the proposal that transmission of excitation from the sympathetic motor nerve initiates action potentials in the muscle cells, and that epinephrine normally acts via a membrane system where depolarization and increase or initiation of spike activity result in contraction.

Although this view has been applied to the vascular muscle, other investigations do not support this general conclusion. Kinetic studies using the rabbit aorta suggest that the adrenergic receptors lie within the muscle cell membrane. Epinephrine and norepinephrine cause contraction of circular smooth muscle in rabbit stomach without producing action potentials or depolarization, and can induce contraction in arterial muscle which has previously been depolarized. Pharmacologically induced contraction has also been described in depolarized visceral muscle. Conversely, the potassium-induced depolarization in the pulmonary artery can be experimentally dissociated from contraction, and in the absence of this dissociation there is a remarkable time lag between these two responses as seen in the present study. It has also been suggested that sodium and potassium are not essential or directly involved in the contraction of rabbit aorta in response to epinephrine. Finally, the contraction of dog carotid artery caused by catecholamines, unlike that by direct electrical stimulation, is not accompanied by a decrease in the arterial potassium content, and this also casts some doubt on the membrane depolarization as an obligatory link in the contractile response to the amines. It appears quite feasible therefore that membrane potential changes are not universally and causally related to muscle contraction. These considerations emphasize that the diversity of properties amongst smooth muscle from various origins demands special caution when generalizations are attempted.
Summary

Simultaneous recordings of isometric tension and muscle membrane potential have been made in the sympathetic nerve-pulmonary artery preparation of the rabbit. The mean resting membrane potential recorded intracellularly was 51.5 mv. Sympathetic nerve stimulation and I-norepinephrine caused contraction without change in the membrane potential and in the absence of action potentials. Increase in extracellular potassium initiated both depolarization and contraction, although these two processes exhibited different latencies and time courses. The significance of these findings is discussed in relation to recent findings of dissociation in smooth muscle between contraction and membrane potential changes.

References

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