THE ELECTRICAL EXCITABILITY OF SMOOTH MUSCLE CELLS

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The responses of smooth muscle to direct electrical excitation differ from those produced by nervous impulses or by the action of adrenaline or acetyl choline. From experiments involving electrical stimulation of the denervated muscles of the nictitating membrane or pilomotor muscles we concluded that these smooth muscles were not electrically excitable [7]. Many authors have studied the responses to direct electrical stimulation of smooth muscles (of the uterus of the rat, or of taenia coli, or of the vas deferens of the guinea pig), and have used microelectrodes [5, 6, 9]. An electrical stimulus applied extracellularly brings about a local contraction beneath the stimulating electrode, and a microelectrode picks up a slow wave of a depolarization. A series of stimuli or a single stimulus of high intensity leads to the development of a critical depolarization, and to the generation of action potentials. However, the slow wave of depolarization and the action potential generated in response to a direct electrical stimulus may be due not to the direct excitation of the membrane of the muscle cells, but to stimulation of the autonomic nervous endings. It seemed interesting therefore to study the cellular potentials under conditions of degeneration of the muscle, so that excitation of the nervous endings would be eliminated.

We have investigated the electrical excitability of the smooth muscle cells of the retractor penis muscle of the dog before and after denervation.

EXPERIMENTAL METHOD

The experiments were carried out on thirty dogs under morphine-urethane anesthesia. The method of preparation of intracellular recording has been described previously [2]. Square-wave stimuli were applied by means of silver electrodes at a separation of 0.2 mm. The stimulating electrode was held in a micromanipulator, which was used to move the extracellular electrode with respect to the tip of the microelectrode. The sympathetic postganglionic nerves were divided beneath the last sacral ganglia of the sympathetic chain. Parasympathetic denervation was achieved by extirpation of the pelvic plexuses. The experiments on denervated muscle were carried out 2-6 days after division of the nerves, i.e., after nervous degeneration [11].

EXPERIMENTAL RESULTS

After the membrane of the smooth muscle cells of an innervated muscle had been pierced the value of the membrane potential did not remain stable. There were slow "spontaneous" oscillations of amplitude about 8-10 mv. Electrical stimulation was applied during a period of comparatively stable membrane potential when "spontaneous" potentials were absent. If the stimulating electrodes were placed further than the 10 mm from the microelectrode contact, then a single electrical stimulus of 2 mseconds duration applied through the stimulating electrode induced a local contraction which could be observed visually under stereomicroscope. The value of the voltage was recorded by a transducer, and was found not to change. In such a case there was no change in the membrane potential either (Fig. 1). When the stimulating electrode was brought to within 7-8 mm of the lead-off microelectrode a slow wave of depolarization in response to the applied stimulus could be recorded. The latent period of this wave in response to isolated stimuli varied from 250 to 550 mseconds, and had an amplitude of 7-10 mv. A reduction of the distance between the electrodes to 1 mm caused the latent period of the slow wave of depolarization to shorten to 150-200 mseconds, but its amplitude remained unchanged. Rhythmical stimulation at a frequency of 2-3 impulses/second caused an increase in amplitude of this wave to a critical value of 17-19 mv, with the result that an action potential was generated. The use of isolated stimuli of 10-15 mseconds duration in some cases also caused an action potential to be generated (See Fig. 1).

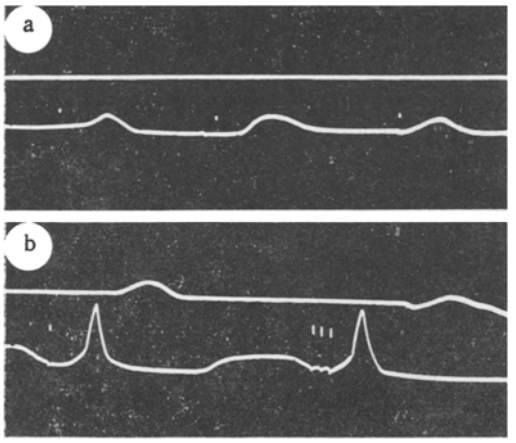

Fig. 1. Potentials from separate smooth muscle cells of an intact muscle in response to extracellular stimulation. a) Show changes of membrane potential; b) action potentials. Curves, from above downwards: voltage of muscle, potentials of separate cells.

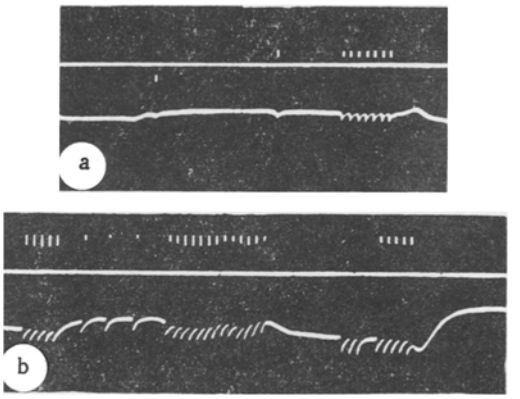

Fig. 2. Responses of separate smooth muscle cells of a denervated muscle to extracellular stimulation. a) Separate stimuli and rhythmical stimulation with a stimulus duration of from 5 to 10 mseconds; b) rhythmical stimulation with a stimulus duration of from 15 to 50 mseconds. Curves as in Fig. 1.

Thus extracellular stimulation in innervated muscle cells causes a slow wave of depolarization which can be recorded at distances up to 10 mm from the site of stimulation. This wave is suggestive of the postsynaptic depolarization observed in cells of the same muscle produced by stimulation of the postganglionic sympathetic nerves [2, 4]. In both cases the duration and amplitude of the membrane potential are closely similar, and an increase of the amplitude and frequency of stimulation produces the same effect, namely, a shortening of the latent period and an increase in the amplitude of depolarization of the membrane.

Stimulation of a denervated muscle with a single stimulus of 2 mseconds duration at an amplitude of 10-15 v with an electrode separation of 12 mm (a stimulus which induces a local contraction in an innervated muscle) gave no effect. Only when the amplitude of the stimulus is increased to 50 v did a local contraction occur beneath the stimulating electrode. The microelectrode records only the artefact of the stimulus (Fig. 2). Reduction of the distance between the electrodes to 1 mm, whether the stimuli are single or repetitive failed to elicit either the slow wave of depolarization or an action potential. Possibly denervation causes changes in the chronaxie of the muscle membrane, and stimuli of duration 2 mseconds do not correspond to the new level of excitability. Therefore, we used stimuli of durations 5, 10, 15, 25, and 50 mseconds, but they too failed to elicit a typical slow depolarization wave and action potential (see Fig. 2). Changes in the amplitude of the membrane potential in response to stimuli of durations 25 or 50 mseconds cannot be interpreted as the development of a typical slow wave of depolarization, because they are produced by a physical spread of the stimulus.

The absence of a slow wave of depolarization and of an action potential when a denervated muscle is stimulated extracellularly confirms the view that both responses elicited by extracellular stimulation of innervated muscle result from excitation of adrenergic nerves. It has been shown [8] that denervation leads to the disappearance of the adrenergic mediator at the sympathetic nerve endings; consequently the absence of a response to direct electrical stimulations appears to be related to the exhaustion of stores of mediator in the degenerating nerve endings.

Evidence for the exhaustion of the mediator through denervation is that in the denervated muscle cell it was impossible to record "spontaneous" miniature potentials which are thought to result from constant secretory activity by sympathetic nerves [1, 3].

We must now consider how to explain why in the normal innervated muscle a slow wave of depolarization and an action potential spread over distances up to 10-12 mm.

According to available data [10] conduction between the smooth muscle fibres is electrical in nature, but for it to occur chemical activity or some related phenomenon is essential; this latter phenomenon sets the level of excitability necessary for the realization of conduction between the cells.

The results of our experiments correspond with these data and indicate that the continuous secretion of an adrenergic mediator is essential, principally in order to establish the level of excitability which makes possible effective conduction between smooth muscle cells. In response to a local electrical stimulus cells of a denervated muscle do not respond by the development of a spreading wave of depolarization and by the appearance of action potentials, but if access to the muscle of a synaptic mediator is prevented the cells lose the ability to respond to electrical stimulation. Contraction of such a muscle, the development of depolarization, and action potentials in normal innervated cells in response to electrical stimulation result from excitation of the postganglionic endings of the sympathetic nerve.

SUMMARY

Microelectrodes were used to study the response of smooth muscle cells of the retractor penis muscle of dogs in response to direct electrical stimulation, before and after denervation. Extracellular stimulation of normal muscles by means of isolated 2-msecond squarewave stimuli induced a slow wave of depolarization which spread over distances up to 10 mm. Repetitive stimulation or stimuli lasting for 10-20 mseconds caused depolarization to attain a critical level, so that an action potential was generated. Extracellular stimulation of a denervated muscle by single or repetitive stimuli induced no slow depolarization or action potential. It is concluded that the denervated cells of the retractor penis muscle are electrically inexcitable. Slow depolarization and action potentials induced by direct stimulation result from the excitation of nerve endings.

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