CHANGES IN MEMBRANE POTENTIAL OF SMOOTH MUSCLES OF THE RABBIT AORTA DURING DEPOLARIZATION BY POTASSIUM CHLORIDE AND SULFATE

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After depolarization caused by KCl the membrane potential was 25 ± 3.6 mV, and after depolarization by K_2SO_4 it was 47 ± 2.9 mV. Synchronous discharges of action potentials with an amplitude of 8-12 mV and frequency of 15-17/min appeared, especially during depolarization produced by KCl.

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Most electrophysiological studies of the smooth muscles of large blood vessels of elastic type have been undertaken with the use of a sucrose bridge [6, 8, 13]. Burnstock and Prosser [6] showed that the membrane potential of the smooth muscles of the pig's carotid artery during depolarization by potassium sulfate is 50 mV. Closely similar values were obtained by Keatinge [8] in experiments on smooth muscles of the sheep's carotid artery, the membrane potential of which was approximately 60 mV during depolarization with potassium sulfate.

In the present investigation changes in membrane potential and contractile reaction of the smooth muscles of the rabbit aorta were studied during depolarization by potassium chloride and sulfate.

EXPERIMENTAL METHOD

The sucrose bridge method suggested for measurement of the membrane potential of a bundle of nerve fibers [11] is widely used for recording the electrical activity of smooth muscles [1, 3, 5, 10]. Modifications of the chamber of the sucrose bridge have been suggested to allow simultaneous recording of the electrical and mechanical responses of smooth muscles [4, 8].

We constructed and used a chamber the design of which is illustrated in Fig. 1. The chamber, made of plexiglass, consists of three separate sections through which runs a horizontal canal 2 mm in diameter into which the strip of blood vessel is drawn. The strip is excised from the thoracic aorta of rabbits (weight 2-2.5 kg) along a spiral in the form of a band 1.5 mm wide and 30 mm long. In the middle section, 10 mm long, the strip of blood vessel is bathed in isotonic sucrose solution made up in deionized water. The resistance of the sucrose solution must be not less than 10^5 Ω/cm. In the section of the left side, also 10 mm long, the strip is bathed in isotonic potassium chloride or sulfate solution. In the right hand section (length 25 mm) the strip of blood vessel is bathed in Krebs' solution of the following composition (in mmoles/liter): NaCl 133, $NaHCO_3$ 16.3, NaH_2PO_4 1.38, KCl 4.7, $CaCl_2 \cdot 6H_2O$ 2.5, $MgCl_2 \cdot 6H_2O$ 0.105, glucose 7.8.

Before entering the right hand section of the chamber the Krebs' solution is aerated with a mixture of 95%O_2 and 5%CO_2 and heated to 35° (Fig. 2). To prevent the solutions from mixing, during assembly the sections of the chamber are separated by sheets of thin rubber into which holes slightly smaller than the thickness of the strip of blood vessel are stamped [1]. The portions of the strip of blood vessel in the side sections are connected electrically through the solutions by means of cotton-wool "tails" with calomel electrodes, connected to the input of a type UI-2 electrometric dc amplifier. The part of the strip of blood vessel lying in the right hand section is fixed to a cork inserted into the lower part of the canal. The right hand section of the chamber is then covered by a detachable lid. The free part of the strip is connected to an apparatus allowing for measured changes in its tension (the weight hanging from the strip is usually 2 g).

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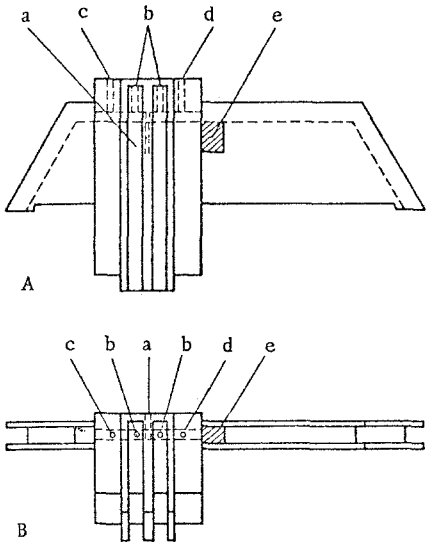

Fig. 1. General view of chamber. A) front view; B) plan a and b) entrance and exit for sucrose solution; c) entrance for solution of potassium chloride or sulfate; d) entrance for Krebs' solution; e) cork for fixing object.

Contraction of the strip of blood vessel is recorded under isotonic conditions by means of a mechanical transducer and a dc amplifier. Electronic potentiometers ÉPP-09 are connected to the outputs of the UI-2 electrometric dc amplifier and the amplifier from the transducer. Changes in electrical potentials and length of the strip were recorded synchronously on the tape of one of the potentiometer to which an additional carriage connected mechanically to the carriage of the second potentiometer was attached.

EXPERIMENTAL RESULTS

During depolarization of the smooth muscles of the rabbit aorta with potassium chloride the membrane potential was 25 ± 3.6 mV, and during depolarization with potassium sulfate 47 ± 2.9 mV. The possible reason for this difference is that depolarization with potassium chloride is incomplete, because in this case chloride ions are present, and according to some authorities [5, 7, 10, 12, 14], this may be of essential importance in determining the membrane potential of smooth muscles.

In some experiments with depolarization by potassium chloride, synchronous discharges of action potentials appeared in the smooth muscles of the blood vessels, their onset coinciding with the development of a contraction (Fig. 3, a). During depolarization by potassium sulfate, no synchronous discharges of action potentials developed in the period when the strip of muscle was bathed by this solution. However, in 2 of the 38 experiments, after perfusion of potassium sulfate had stopped, the appearance of synchronous discharges of action potentials, essentially indistinguishable in amplitude and frequency from the action potentials evoked by depolarization with potassium chloride, was observed through the right hand section of the chamber. In these rare cases, however, the appearance of synchronous discharges of action potentials did not coincide with the onset of contraction, because they appeared against the background of an established contraction.

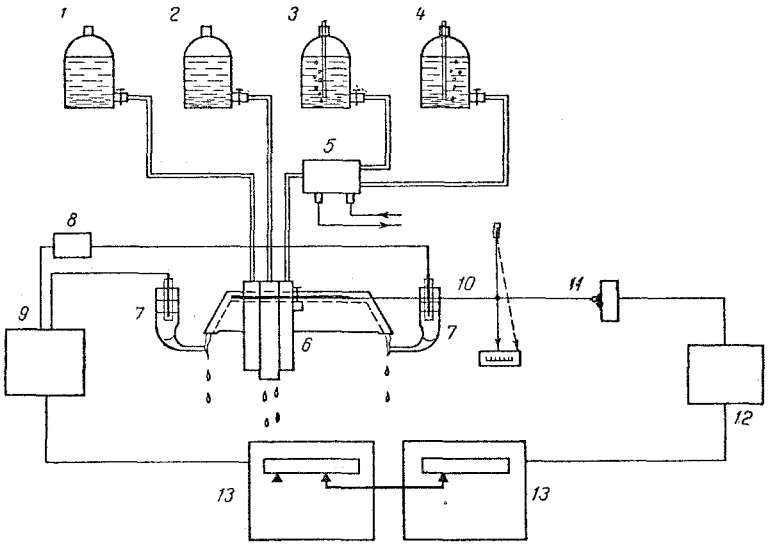

Fig. 2. Diagram of apparatus for simultaneous recording of electrical and mechanical response of smooth muscles of rabbit aorta. 1, 3) Isotonic potassium chloride (potassium sulfate) solution; 2) isotonic sucrose solution; 4) Krebs' solution; 5) heat exchanger; 6) chamber of sucrose bridge with object; 7) calomel electrodes; 8) calibrator; 9) UI-2 electrometric dc amplifier; 10) device for measuring change in tension of object; 11) mechanical transducer; 12) dc amplifier; 13) ÉPP-09.

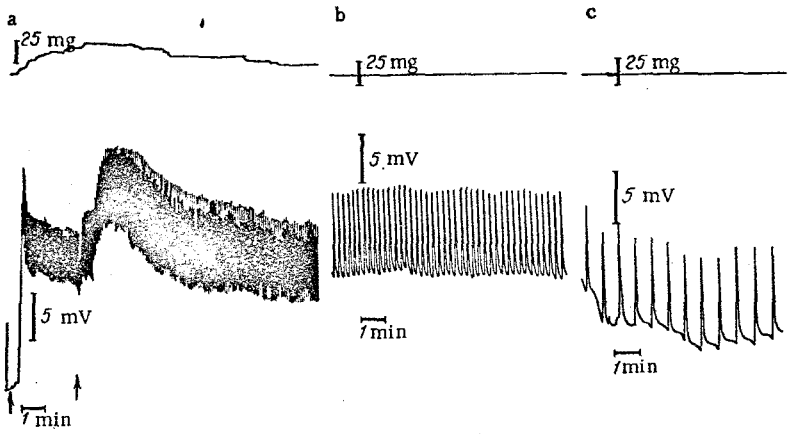

Fig. 3. Mechanical response and appearance of synchronous discharges of action potentials in smooth muscles of rabbit aorta during depolarization with potassium chloride (a) and gradual slowing of rhythm of synchronous discharges of action potentials after 62 min (b) and 114 min (c). From top to bottom: trace of contraction, trace of changes of membrane potential. Arrows indicate beginning and end of action of potassium chloride.

After the action of potassium chloride and sulfate had stopped, synchronous discharges of action potentials were observed for 2.5 h, despite the fact that relaxation of the muscle took place after 20-30 min. In most cases the frequency of the action potentials was gradually reduced in the course of 1.5-2 h, from an initial 15-17 discharges to 2-3 discharges/min (Fig. 2, b, c). The configuration of the action potentials also changed mainly on account of slowing of the repolarization phase. The amplitude of the action potentials was essentially unchanged, remaining at 8-12 mV. Sometimes the amplitude of these potentials increased to 30 mV shortly before the end of spontaneous electrical activity, while their duration reached 1 min on account of the appearance of a repolarization plateau [2].

In some experiments, instead of gradual slowing of the action potentials for a period of 1.5-2 h, an abrupt, stepwise transformation from one rhythm to the other was observed, the frequency varying over a period of several minutes from 15 to 3-4 discharges of action potentials per minute. Usually this transition was associated with a sudden increase in level of the membrane potential.

In the smooth muscles of the rabbit aorta, depolarization by potassium may thus give rise to synchronous discharges of action potentials which are observed for a long time, even after the smooth muscles have become completely relaxed. Keatinge [9] recorded fast rhythmic electrical activity in the smooth muscles of the sheep's carotid artery. The amplitude of these action potentials reached 6-8 mV and their frequency 12-14/min. In his experiments, however, rhythmic activity developed only after the strip of blood vessel had been kept for about 30 min in calcium-free Krebs' solution.

In our experiments, in contrast to those of Keatinge, when the bioelectrical activity of the vascular smooth muscles was recorded by the sucrose bridge method, the appearance of synchronous discharges of action potentials was observed during depolarization with potassium chloride, but less frequently by potassium sulfate in the presence of calcium ions. The reason for this difference is not clear.

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