SIMULTANEOUS RECORDING OF CHANGES IN THE MEMBRANE POTENTIAL AND TONE OF SMOOTH MUSCLE IN VITRO

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The changes in the membrane potential of smooth-muscle cells can be recorded intracellularly by means of glass microelectrodes, extracellularly with very slight compression of the cells by the microelectrode with a point of comparatively large diameter [6, 7], and extracellularly by the sucrose bridge method [1, 2, 3, 5]. The use of the first method is restricted by the small diameter of the smooth-muscle cells (from 3 to 6 μ), making it impossible to obtain long records from one cell. The second and, in particular, the third methods have considerable advantages in this respect over intracellular recording. By means of the sucrose bridge method, prolonged observations can be made on the changes in the electrical activity of the smooth muscle for as long as several hours. The principle of the method is the creation of a high external impedance of an area of the smooth muscle by means of a high-ohmic isotonic solution. The fluid used for this purpose for warm-blooded animals is a 10% sucrose solution, replacing the extracellular fluid between the muscle fibers if perfusion is continued long enough. If the recording electrodes are applied to both sides of the area of muscle perfused with sucrose solution, extracellular recordings can be made of the changes in the membrane potential of the smooth-muscle fibers. The absolute magnitude of the membrane potential can be determined by using what is known as the short-circuit factor [4].

However, the variants of the sucrose bridge method which are in use are not without their disadvantages, and these are reflected in the conduct of the observations. Bülbring and Brunstock [3], for example, do not rule out the possibility that irregular displacement of the sucrose solutions and nutrient fluid may occur even in the presence of very small movements of the smooth muscle in isometric conditions. The electrolyte thereby enters the sucrose solution and introduces an artefact into the recording. In other variants of this method [1, 2] no provision is made for the simultaneous recording of the mechanical activity of the muscle. In addition, the design of the chamber used by D. P. Artemenko and M. F. Shuba [1] requires high-speed perfusion with sucrose solution (up to 20 ml/min) if all the electrolyte is to be washed out of the muscle, and large amounts of sucrose are therefore essential.

The author has used a chamber of comparatively simple construction yet enabling simultaneous recordings to be made of the electrical and mechanical activity of the smooth muscle, and avoiding the need for mixing the nutrient fluid with sucrose solution.

The chamber is made of organic glass and consists of three sections as indicated in Fig. 1a. The diameter of the horizontal channel in the middle section is 1.5 mm and its length 10 mm. The diameter of the narrow part of the vertical channel meeting the horizontal is 1 mm. The diameter of the vertical channels in the side sections is 2 mm. The vertical channels in sections A and C stand 1 mm away from the ends of the horizontal channel of sec- B. The diameter of the oblique channel in section C, through which the nutrient fluid or the test solution enters, is 1 mm. Polyethylene tubes of suitable diameter to allow the corresponding solutions to enter the chamber are inserted into the upper parts of the vertical channels in sections A and B and into the outer part of the oblique channel of section C. During assembly of the chamber thin rubber sheet, with holes cut out at the level of the horizontal channel of the middle section, is placed between the sections. Threads are introduced into the vertical channels of sections A and C, with "brushes" of cotton wool fixed to their ends. The segment of muscle (in the author's experiments the taenia coli of a guinea pig), 1.5-2 cm in length, with silk threads tied to it at both ends, is then passed through the hole in the rubber sheet and through the horizontal channel of section B and the hole in the other rubber sheet. The diameter of these holes must be slightly less than the diameter of the muscle, so as not to compress the muscle fibers too much. The free ends of the muscle are then inserted into the vertical channels of the side sections. One end is tied by means of the silk thread to the tube through which the nutrient fluid enters, while the other is connected to a comparative pick-up for recording the muscle tension. The system is shown schematically in Fig. 1b. After the system has been assembled perfusion with all the solutions at a constant speed is established.

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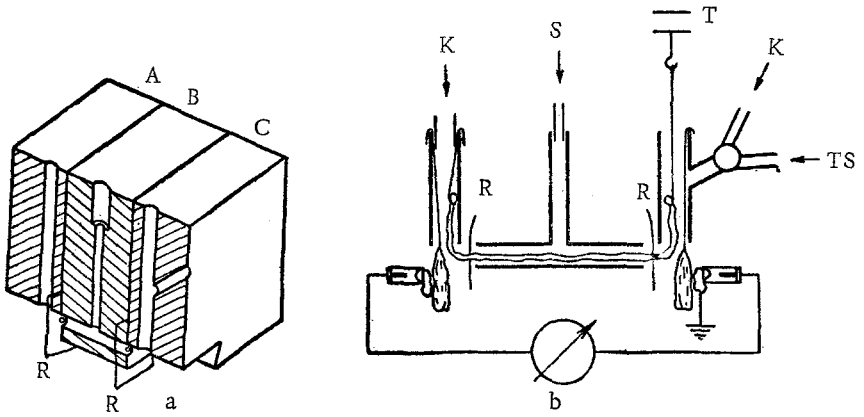

Fig. 1. Section through chamber for recording the electrical activity of the smooth muscle by the sucrose bridge method (a) and a scheme showing the assembled system (b). R) Rubber dividing membrane; K) Krebs' solution; TS) test solution; S) sucrose solution; T) tensiometric pick-up.

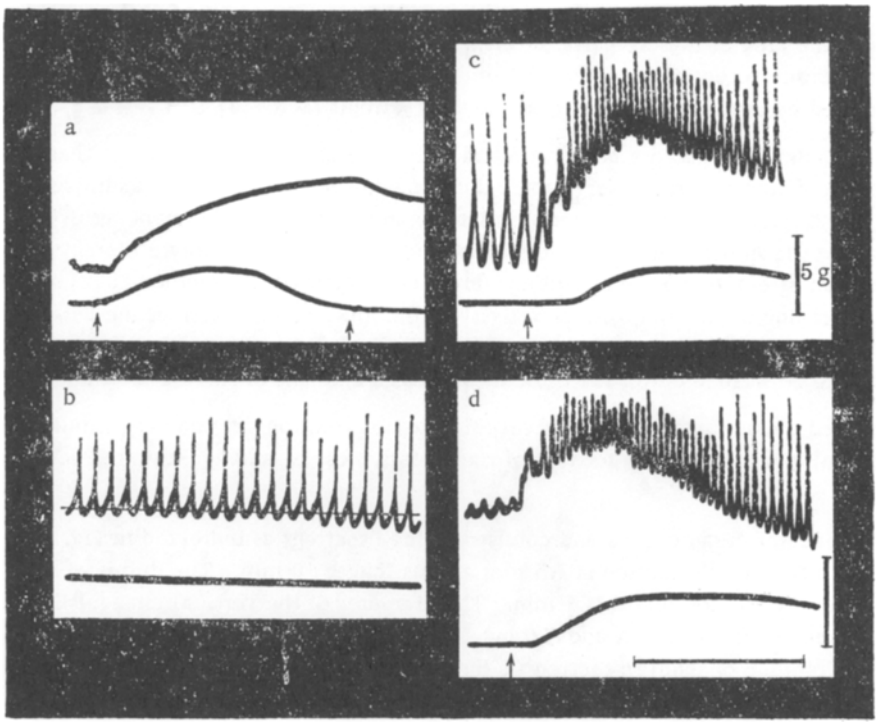

Fig. 2. Character of electrical and mechanical activity of the taenia coli of a guinea pig. a) During action of potassium sulfate solution (127 mmole) in Krebs' solution (the arrows indicate the beginning and end of perfusion with the test solution); b) spontaneous action currents; c) during the action of acetylcholine (10^{-5}) against the background of spontaneous action currents; d) the same in the absence of this background (the arrows indicate the times of addition of acetylcholine). From top to bottom: electrical activity, mechanical. An upward deflection of the beam corresponds to depolarization and to an increase in the tone of the smooth muscle. Temperature 32°. Calibration signal for tracing:a 50 mV, for b 10 mV, and for c and d 5 mV. Time marker for tracing:a 50 sec, and for b, c, and d 20 sec.

The rate of flow of each fluid is about 2-3 ml/min. The sucrose solution and the nutrient fluid or the test solution entering the section C are warmed to the required temperature, measured in section C by means of a thermocouple. The temperature of the nutrient fluid entering section A is kept at about 10-15° to depress the activity of that part of the preparation in section A. The solutions entering section C are saturated with oxygen. The test solutions can be changed by means of a two-way tap as close to the chamber as possible.

With very slight movements of the muscle it carries the rubber membranes with it and so prevents the part of the muscle fibers bathed in electrolyte solutions from entering the sucrose solution. The use of the rubber membranes in the chamber thus prevents the development of artefacts connected with entry of the electrolyte into the sucrose solution flowing through the horizontal channel of section B. In the author's experiments a 10% solution of sucrose (All-Union State Standard 5833-54) in bidistilled water was used. The resistance of this solution was more than $5 \cdot 10^5$ ohm/cm, and there was therefore no need for further purification of the solution on an ion-exchange resin.

The potential difference between the junctions of the sucrose solution and the nutrient (or test) fluid were recorded with a dc amplifier, connected through a cathode repeater and nonpolarizing Ag—AgCl electrodes to the cotton-wool "brushes" projecting from the vertical channels of the side sections of the chamber. The signals from the screen of the two-channel oscillograph were recorded on a stationary motion picture film. Some types of changes in the membrane potential and the tension of a smooth muscle recorded by the method described are illustrated in Fig. 2.

SUMMARY

A modification has been described of the sucrose gap method for recording resting and action potentials simultaneously with the registration of tension in the smooth muscle.

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