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ELECTRICAL PROPERTIES AND TRANSMEMBRANE IONIC CURRENT OF SINGLE SMOOTH MUSCLE CELLS

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The study of the electrophysiological properties of smooth muscles is beset with great technical and analytical difficulties, due to their distinctive structural features. The small size of smooth muscle cells (SMC), the presence of electrical connection between them, and also their contractile activity are a substantial handicap for the effective use of microelectrode research techniques. Meanwhile the good electrical connection between SMC does allow the sucrose gap technique to be used for the study of their electrophysiological properties. However, because of the disadvantages inherent in this method (interdiffusion of solutions flowing through adjacent compartments, the presence of diffusion potentials and of stray bypass circuits) the possibility of quantitative analysis of the results obtained by its use is substantially limited [1]. These factors, and also difficulties in creating adequate conditions for the investigation of transmembrane currents by the voltage clamp method on multicellular smooth-muscle preparations, have acted as a stimulus for the search for possible ways of utilizing single SMC in electrophysiological research. By now several investigations have been published in which attempts have been made to study passive and active electrical properties [7-9] and also transmembrane currents [11] of single SMC, with the use of intracellular microelectrodes.

This paper describes the results of such investigations, using a single suction micropipet [3], which has significant advantages over the microelectrode method [3, 4].

EXPERIMENTAL METHODS

Experiments were carried out on freshly prepared myocytes on the guinea pig taenia coli and ileum. To obtain isolated cells, small pieces of muscle tissue were kept for 10-15 min in calcium-free Krebs' solution, and then subjected to enzyme treatment (30 min, 36°C, pH 7.3, without mixing) in a solution containing (in mM): NaCl 120.4, KCl 5.9, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11.5, to which were added 1 mg/ml of collagenase (type I, from Sigma, USA), 1 mg/ml of bovine albumin, and 0.5 mg/ml of soy trypsin inhibitor. Pieces of tissue were then transferred into a solution with composition resembling that of "KB medium" [6] and containing (in mM): KCl 85, KH_2PO_4 30, $MgSO_4$ 5, Na_2ATP 5, creatine 5, glucose 20, EGTA 3 (the pH of the solution was adjusted to 7.2 with KOH). The pieces of tissue in this solution were repeatedly passed through a Pasteur pipet until a cell suspension was obtained. After 1 h a drop of suspension was added to an experimental chamber with a volume of 0.1 ml, located in the field of vision of an inverted microscope, and filled with Krebs' solution. After 3-5 min the myocytes easily adhered to the glass bottom, so that the chamber could be perfused with normal or testing Krebs' solution, previously filtered through a filter with pore diameter of 0.5 μ. Perfusion of the chamber was stopped during recording. The experiments were carried out at room temperature. The procedure of preparing and filling the glass micropipets, and also of obtaining high-ohmic contact with the membrane surface was identical with that described previously [3]. The micropipets used in the experiments had a resistance of 1-2 MΩ and were filled with a solution of the composition mentioned above ("KB medium").

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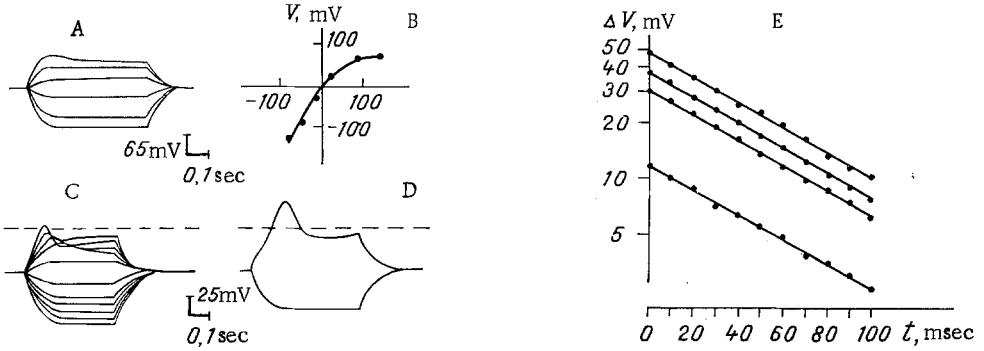

Fig. 1. Passive and active electrical responses of single SMC of taenia coli to square pulses of hyper- and depolarizing current. A, B) Electrotonic potentials and current-voltage characteristic curves illustrating the rectifying properties of the myocyte membrane; C, D) electronic potentials and gradual AP in normal (C) and barium-containing (D) Krebs' solutions. Broken line — zero level of transmembrane potential; E) illustrations of monoexponential character of rise of anelectrotronic potentials, indicated in Fig. 1C. Ordinate, value of $\Delta V = V_{max} - V$, on a logarithmic scale, where V denotes membrane potential at time t, V_{max} denotes steady-state value of anelectrotonic potential.

An Ag-AgCl electrode, in contact with the solution in the micropipet, under current clamping conditions was connected with the current generator and voltage follower with high input resistance and compensated leakage current. This last feature is of great importance because the myocytes investigated had high input resistance, namely 0.8-0.3 GΩ.

Under voltage clamp conditions the holding and command potentials were applied through the micropipet and the transmembrane current was measured by means of an operational amplifier, connected to an indifferent electrode, whose potential was maintained at zero. The resting potential (RP) of the myocytes was determined from the change in potential during penetration of the membrane under current clamping conditions.

EXPERIMENTAL RESULTS

Relaxed myocytes with no appreciable signs of injury, whose RP exceeded −40 mV and whose input resistance was not less than 800 MΩ, were chosen for the electrophysiological investigations. When making the choice, the following considerations served as the guide: 1) high input resistance and near-normal RP of the cells are evidence that the metabolic mechanisms maintaining transmembrane ionic gradients and membrane conductance were not damaged by enzymic and mechanical treatment of the myocytes; 2) high input resistance of the cells with low after-resistance of the micropipet, which was 3-5 MΩ after perforation of the membrane, enables the use of a second intracellular electrode to be dispensed with, under both current and voltage clamp conditions, without detriment to accuracy of measurement; 3) high input resistance of the myocytes suggests that their membrane can be regarded with a considerable degree of accuracy as an isopotential surface, even during activation of potential-dependent conductances [8, 9]. Estimation of the spatial constant of single SMC by means of the equation for a short cable [12] gives a value of about 3 mm, which is an order of magnitude higher than the length of the muscle cell. Under these circumstances the potential at the end of the cell differs from the potential at its center by under 1%. This is of fundamental importance for determination of specific values of capacitance and resistance of the membrane, for under isopotential conditions the equivalent electrical circuit of the membrane of a single muscle cell can be represented as a parallel RC-circuit. In turn, this suggests an exponential rise and fall of electrotronic potentials and it can be tested experimentally. The results of experiments of this kind are shown in Fig. 1.

Under the influence of small pulses of hyperpolarizing currents, the rise and fall of the electrotonic potentials could be approximated actually by one exponential function (Fig. 1E), the time constant of which varies in different cells from 90 to 240 msec. Variations of the time constant were connected with variations of the specific resistance of the myocyte membrane, which was determined by multiplying the input resistance of the cell by its surface area. During determination of the time constant, consideration was paid to bypassing of the

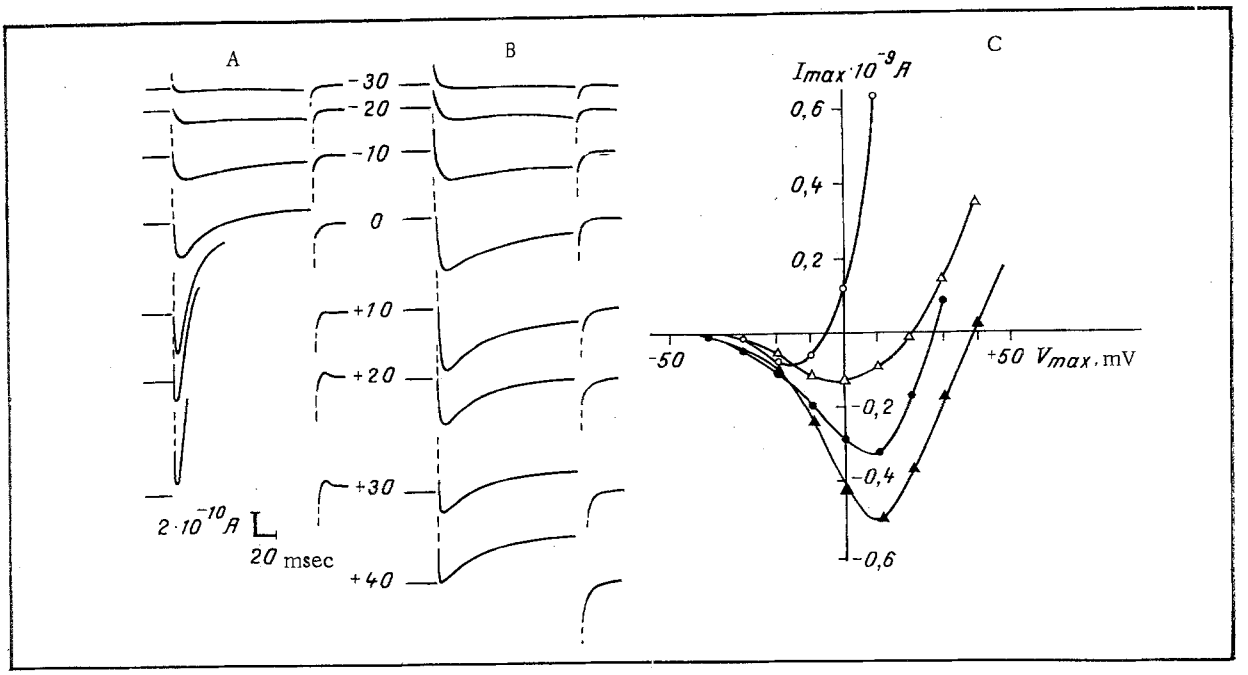

Fig. 2. Transmembrane ionic currents (A, B) and current-voltage characteristic curves (C) of myocytes of ileum in normal Krebs' solution (A) and under the influence of 10 mM TEA (B). Filled circles and triangles indicate maximal amplitude of input current, empty circles and triangles represent steady-state value of transmembrane current in normal solutions and under influence of TEA respectively. Numbers near traces of current show level to which transmembrane potential was shifted during their recording. I_{max} and V_{max} denote peak values of current strength and voltage.

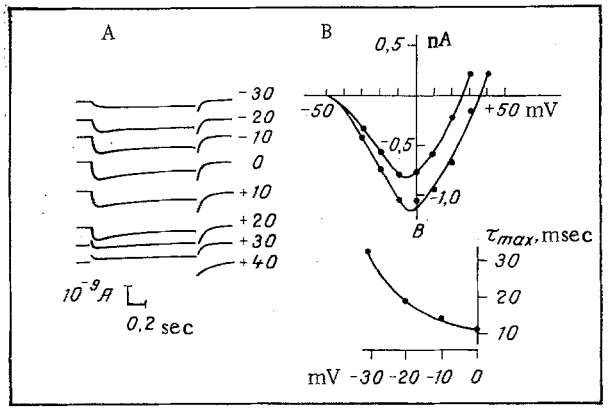

Fig. 3. Ba currents through Ca channels of SMC membrane from the ileum. A) Traces of transmembrane currents; B) current-voltage characteristic curves for peak of inactivated (bottom trace) and stationary (top trace) components of Ba-current; C) dependence of activation time constant (τ_m) of inward current on transmembrane potential.

membrane by the output resistance of the current generators, which in this series of experiments was 1 GΩ. The specific capacitance of the membrane, calculated from the time constant and specific resistance, varied within narrow limits. If the visible dimensions of the cell were adopted for calculating surface area, and its shape was approximated by the contacting bases of two cones, the specific capacitance of the membrane was calculated to be on average 2.7 ± 0.2 μF/cm², and the specific resistance in different cells could vary from 30 to 90 kΩ·cm². However, the very large number of invaginations of the membrane (caveolae) can significantly increase its surface area. For example, for taenia coli cells, the gain in area due to caveolae may amount to 70% [2]. Allowing for this correction, the specific capacitance

of the membrane is reduced to 1.6 μF/cm^2. This value is twice as high as that for isolated cells of the frog stomach [8], and it is close to the value for these same cells, calculated from the time constant of rise of the base of the spreading action potential (AP) [10]. Allowing for this correction, the specific resistance varied from 50 to 150 kΩ·cm^2. The current-voltage characteristic curve of SMC was linear during a shift of the transmembrane potential toward hyperpolarization (Fig. 1B). For depolarizing currents the membrane exhibited rectifying properties (Fig. 1B, C). As a rule an active response, reminiscent of a local potential, was observed on a catelectrotonic potential (Fig. 1A). Muscle cells of the taenia coli generated a gradual, delayed AP, much less frequently; its amplitude and rate of rise depended on the strength of the stimulating current (Fig. 1C). The absence of regenerative AP, characteristic of this type of smooth muscle, was evidently connected with the bypassing action of the output resistance of the current generator, whose value was of the same order of magnitude as the input resistance of the cell, and also with the fact that the experiments were conducted at room temperature. The possibility of destruction of some of the Ca channels, which are responsible for AP generation in SMC of the taenia coli [5], during enzyme treatment likewise cannot be ruled out. Indirect evidence of this is given by results obtained on myocytes of the frog stomach, which generated AP only after the external Ca^{++} ion concentration had risen about 8 mM [8]. In the present experiments replacement of Ca^{++} ions by 10 mM Ba^{++} ions, for which Ca channels are more permeable, also facilitated AP generation (Fig. 1D). Under these circumstances the amplitude and duration of AP increased, and this also is partially connected with the blocking action of Ba^{++} ions on K-conductance.

Transmembrane currents recorded in single muscle cells of the taenia coli and ileum were qualitatively similar to currents recorded on multicellular muscle preparations under double sucrose gap conditions. However, the use of a suction micropipet instead of a sucrose gap enabled absolute values of the transmembrane currents and potentials to be measured. In addition, due to the low after-resistance of the micropipet the time constant of the capacitive currents was 0.2-0.3 msec, so that the kinetics of activation of the inward current could be investigated quantitatively. During the study of single cells influences of narrow intercellular spaces, in which the concentration of current-carrying ions may vary significantly during activation of the ionic conductances of the membrane, also are are ruled out. Transmembrane currents of a single myocyte of the guinea pig ileum in normal Krebs' solution and after addition of 10 mM tetraethylammonium (TEA) are shown in Fig. 2. With small depolarizing shifts of transmembrane potential the inward current consisted of two components: inactivated and stationary. With an increase in depolarization, the decline of the inward current was accelerated because of the ever-increasing contribution of the outward current. At a voltage of 5 mV the stationary current, measured at the end of the testing pulse, was reversed. The maximal value of the inactivated component of the inward current was observed at a voltage of +8 mV. This value varied in different cells from 0 to +10 mV. The rate of activation of the inward current depended on the magnitude of the depolarizing shift and increased with an increase in depolarization.

Under the influence of TEA the amplitude of both the inactivated and the stationary component of the inward current increased considerably, but the reversal potential was shifted to the right along the voltage axis (Fig. 2B, C). Under these circumstances activation of the inward current was accelerated. This is evidence of early activation of the outward current and its superposition on the inward current within a narrow time interval. However, complete blocking of K conductance could not be achieved by means of TEA ions, even after a considerable increase in their concentration.

On replacement of Ca^{++} ions by 10 mM Ba^{++} the inward currents were substantially increased and their activation kinetics delayed (Fig. 3). The increase in the stationary inward current was particuarly marked, and in normal solution it was considerably masked by the outward K current. The maximum of the current-voltage characteristic curve for Ba currents was shifted toward more negative potentials compared with that for Ca currents; this was evidently connected with differences in the affinity of binding of these ions with the surface charges on the membrane. Activation of the inward barium current obeyed an exponential rule. This suggests that the contribution of the outward potassium current to the transmembrane current carried by Ba^{++} ions is negligible. The character of the potential-dependence of the activation time constant of Ba currents through Ca channels (Fig. 3C) was approximately the same as for Ca currents. However, the considerable slowing of activation of Ca channels in Ba solution is evidence that this process depends not only on potential, but also on the type of ion passing through the channel.

On the basis of the results obtained by the study of transmembrane currents in the presence of TEA, we estimated the increase in the intracellular Ca^{++} concentration created by the inward Ca current during excitation of the cells. This assessment was based on the volume of the myocytes, calculated from their visible dimensions, and the quantity of electricity carried by the inward current during a shift of membrane potential of magnitude and duration comparable with the corresponding parameters of AP. The intracellular Ca^{++} concentration calculated in this way was 10^{-5} M, which, even allowing for possible partial intracellular binding of Ca^{++} ions entering the cell, is sufficient to activate contraction without any additional release of Ca^{++} from the intracellular storage depots.

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