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"MICROPIPET" METHOD OF RECORDING FAST INWARD IONIC CURRENTS OF SINGLE HEART MUSCLE CELLS

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Recently developed techniques for working with single heart cells provide an approach to the problem of creating an effective voltage clamp system for measuring fast ionic currents [2, 4, 6]. To obtain reliable voltage clamping and adequate resolutions between the capacitive current and the initial phase of the fast ionic current, it was decided to use the idea of the suction micropipet for recording the integral currents from a small area of the membrane of a single heart cell.

EXPERIMENTAL METHOD

Single heart cells were isolated by the method in [8]. The heart was removed quickly from rats weighing 200-300 g under ether anesthesia. The heart was then perfused through the aorta under a pressure of 90 cm water with a standard solution of the following composition: NaCl 140 mM, KCl 5.4 mM, $MgCl_2$ 1 mM, glucose 11 mM, Tris 10 mM, human serum albumin (from Reanal, Hungary) 1 mg/ml (pH 7.4). The temperature of the solution was 37°C. The solution was saturated with oxygen. Collagenase (type 1, from Sigma, USA) and $CaCl_2$ to final concentrations of 0.4 mg/ml and 25 μM respectively were added 4 min later to the perfusion fluid and perfusion continued for 15 min. The ventricles were then cut off and placed in 40 ml of a standard solution containing 0.2 mM $CaCl_2$. The tissue was then cut into pieces with scissors and the resulting cell suspension was filtered through nylon gauze. After sedimentation of the cells the supernatant was carefully poured out and replaced by 40 ml of a fresh standard solution containing 0.9 mM $CaCl_2$.

A cell chosen under the microscope (magnification 155) was transferred by means of a polyethylene pipet into the working chamber. The cell was then fixed in the pore of a V-shaped suction pipet [1] (diameter of pore 5-6 μ), in which a pressure of 50 cm water was created. Both the chamber and suction pipet contained standard solution with 0.9 mM $CaCl_2$. The experiments were carried out at room temperature (20-22°C).

A scheme of the experimental arrangements is shown in Fig. 1A. A command potential is applied to electrode E1. The signal on electrode E2 is held at the zero level by means of a

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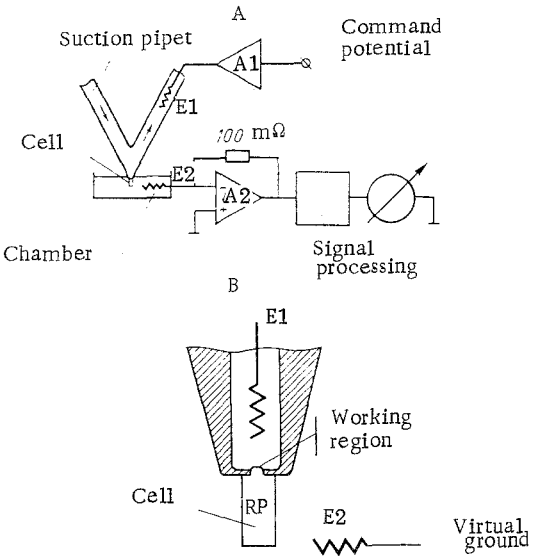

Fig. 1. Scheme of experimental arrangements (A) and of cell held in pore of suction pipet (B). A: A1) Command signal shaper, A2) amplifier of current–voltage converter. E1 and E2) Ag/AgCl electrodes; "signal processing" activates leakage current subtractor, restoration of high-frequency components of signal, and active filters at frequencies of 2.5, 5, 10, and 15 kHz to filter background noise; B: RP) resting potential of cell. Command potential applied to E1, potential held at zero level on E2 by current–voltage converter.

"virtual ground" circuit. A potential equal to the difference between the resting potential of the cell and the command potential is thus established on the test region of the membrane (Fig. 1B). Primary processing of the recorded signal is indicated in the scheme: subtraction of the leakage current, restoration of the high-frequency components of the signal, and active filtration of the signal.

EXPERIMENTAL RESULTS

The basic idea of the method is that the resistance of the test region of the membrane was 200-300 times greater and its capacitance 200-300 times less than the resistance and capacitance of the rest of the membrane (corresponding to their relative areas). Under static conditions practically all (with an accuracy of 1/200-1/300) command potential falls on the resistance of the working region. The dynamic regime (establishment of the command potential, development of the ionic current) was analyzed analytically and by computer, taking into account the full frequency-dependent impedance of the nonworking part of the membrane. Ionic currents were described by equations of the Hodgkin–Huxley type [5]. The passive characteristics of the membrane were taken from [8]. The following observations were made: 1) The potential on the working region of the membrane was established with an error of 0.3-0.5% for a period of not more than 10 μsec; 2) the faster the ionic current developed, the less the deviation of the membrane potential from its assigned value (clamping error); 3) at currents whose amplitude did not exceed 350 pA at any voltages, the "measured" current (calculated with allowance for after-impedance) was virtually indistinguishable from the "ideal" (without after-impedance); 4) peak values of the currents were "recorded" virtually without error within the amplitude range up to 500 pA.

The shape of the current-voltage relationship of the peak currents is one criterion of the quality of voltage clamping (Fig. 2). In the present experiments "collapse" of the current was never observed during plotting of the descending part of the current–voltage relationship. Potentials counted from the resting potential of the cell, which was not measured in the present experiment, are plotted on the abscissa in Fig. 2. Comparison of

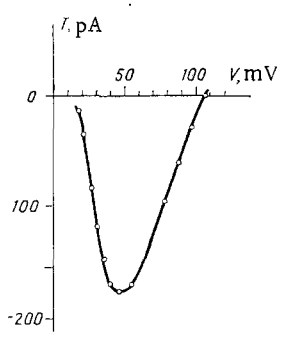

Fig. 2

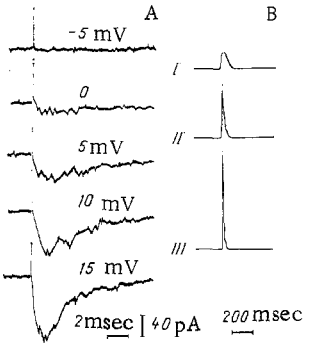

Fig. 3

Fig. 2. Current–voltage relationship of peak rapid inward current. Abscissa, potentials counted from cell resting potential. Holding potential –20 mV from resting potential.

Fig. 3. Superposed traces of fast inward currents (A) and experimental traces of "capacitance currents" (B). A: Filtration band 10 kHz, numbers above curves correspond to potentials counted from cell resting potential. Holding potential –40 mV from resting potential; B: I) filtration band at output 5 kHz, II) 15 kHz; III) after partial restoration of high-frequency components of signal.

amplitudes of the currents in response to the same pulse at different stages of the experiment was used as the test for stability of the cell resting potential. In most cases the amplitude of this current was virtually unchanged during 1.5-2 h of the experiment.

Traces of ionic currents with subtraction of the leakage current are given in Fig. 3A. The traces clearly show an increase in the fluctuations during development of the ionic current. For the purpose of demonstration an experiment was chosen in which the diameter of the test region was small and fluctuations were most clearly evident. It must be assumed that these fluctuations were connected with the statistical character of functioning of the ionic channels. Background noise was determined mainly by the heat noise of the resistor shunting preparation along the walls of the suction pipet: Its value lay within the range 20-60 MΩ. The greatest mean-square value of this noise (see the regions of the traces before application of the stimulus), calculated by the equation $\langle \Delta I^2 \rangle^{1/2} = \sqrt{4kTB/R_L}$, where B is the frequency band of the observed noise ($B \leqslant 10$ kHz) and R_L is the resistance of the shunt, did not exceed 4 pA, appreciably less than the observed fluctuations.

To suppress the outward currents $BaCl_2$ was used [3]. After addition of $BaCl_2$ in a concentration of 1 mM the outward currents were not recorded. On the addition of tetrodotoxin to the solution in a concentration of 4×10^{-6} g/ml the inward currents were considerably reduced, but a total block was not observed.

To ensure a satisfactory signal to noise ratio in the feedback of the amplifier A2 (Fig. 1A), a resistance of 100 MΩ was included. Because of stray capacitance of the order of 0.25 pF in this resistor, due to its design, the transmitted signal was filtered with a time constant of 25 μsec. Special analysis showed that this filtration had no effect on the quality of potential control at the input of amplifier A2. When the command potential is switched to electrode E1 (in our case, after 0.5 μsec) the potential on electrode E2 was established at the zero level in the course of about 3 μsec, the single amplification bands of amplifier A2 being 15 MHz. The transition process recordable for a long time at the input of the current–voltage converter (the "capacitive current" with a duration of 70-75 μsec), masked the initial phase of development of the current at high voltages. However, the potential on the membrane was established sufficiently quickly, so that the ionic current reached the input of amplifier A2 undistorted and was subsequently filtered by linear elements. The distortions arising under these circumstances could be corrected in the next cascade by raising the amplification factor for high frequencies. In this way we were able to adjust the duration of the recordable transition process to 30 μsec. Original traces of the "capacitive current" are given in Fig. 3B.

If the diameter of the test region was about 5 μ the population of ionic channels, on the one hand, was small enough for fluctuations of ionic current to be clearly visible, and on the other hand, it was sufficiently large for integral ionic currents to be recorded with a single sweep of the beam. This combination of circumstances makes the method well suited for the study of ionic channels by both classical and statistical methods of analysis.

When the technique of micropipet recording is used, it will be noted, the cell remains uninjured. This means that the life of the cell can be maintained for a long time in the course of an experiment — on average 1.5-2 h.

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