PHYSIOLOGY

ACTIVATION OF THE FAST TRANSMEMBRANE SODIUM CURRENT OF SINGLE HEART CELLS

Yu. I. Zil'berter and E. N. Timin

UDC 612.173.1.014.461.2

KEY WORDS: patch voltage clamp method; single heart cell; fast sodium current; activation.

Ability to measure parameters of the activation of the fast sodium current is determined by the time of reversal of the membrane potential. In previous studies [3-5], when characteristics of activation were measured in single heart cells, the time constants of membrane potential reversal were 80-100 μsec, and consequently, the capacitive current masked a large part of the ascending phase of the sodium current at high depolarizing potentials. Passive subtraction of the capacitive current [4] cannot improve the situation, for the sodium current develops during the first 200-300 μsec while the membrane potential is changing.

The writers previously [2] described a microrecording method enabling the membrane potential to be reversed for not more than 10 μsec. This time resolution enabled the ascending phase of the sodium current to be reliably distinguished over a wide range of membrane potentials.

EXPERIMENTAL METHODS

Cells were isolated by the method in [8] and the experimental procedure was described previously [2]. The compositions of the solutions were described in [1]. The experiments were performed at room temperature (20-22°C).

The currents were measured by means of a "virtual ground" circuit. Signal distortion on account of filtration as far as the 3 kHz band on stray capacitances of the measuring channel were corrected to the 40 kHz band by means of a circuit restoring the high-frequency components of the signal. The signal was isolated from noise by the method of synchronous cumulation on an ATAC-250 averager (Nihon Kohden, Japan). In all experiments the membrane potential was set relative to the resting potential of the cell.

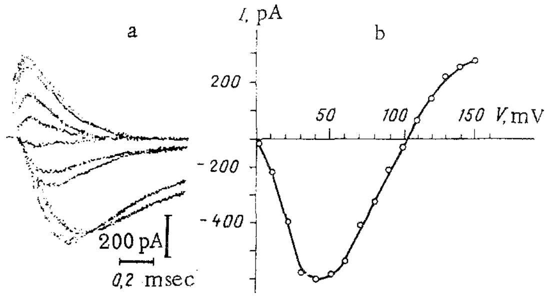

Fig. 1. Original recordings of fast sodium currents (a) and current—voltage characteristics of peak values of current (b). a) Currents recorded by synchronous cumulation method (ten summations) after restoration of high-frequency components. Membrane potentials: 40, 50, 80, 90, 100, 110, 120, 140, and 150 mV.

Laboratory of Biophysical Research, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 3-4, November, 1982. Original article submitted May 28, 1982.

0007-4888/82/9411-1465$07.50

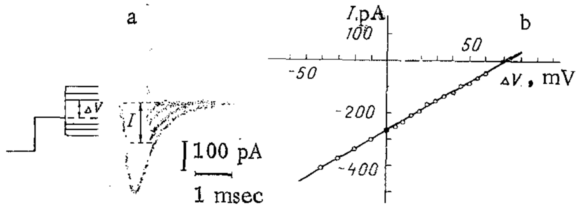

Fig. 2. Momentary current—voltage characteristics. a) pulse program (on left) and original recording of sodium currents (right). Conditioning pulses, amplitude 105 mV, were applied from a holding potential of —50mV; b) abscissa, steps of potential (in mV, see a); ordinate, values of current (in pA) in response to reversal of potential. Filled circle — current at moment of potential reversal.

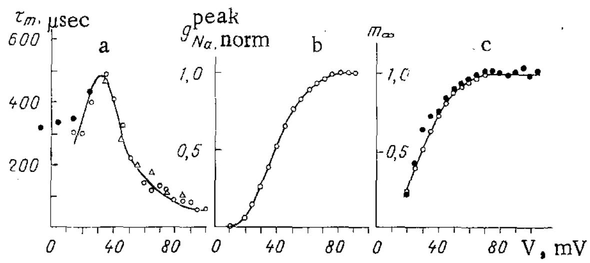

Fig. 3. Time constant of activation (a), peak values of sodium conductance (b), and steady-state activation level (c) as functions of membrane potential. a) Different symbols correspond to results of different experiments. Continuous curve drawn by eye through results of one experiment (empty circles); b) g_{Na}^{peak} normalized against its maximal value; c) empty circles — results of calculation for peak conductance, filled circles — for results of extrapolation of descending phase of current to origin of time axis.

EXPERIMENTAL RESULTS

Superposed recordings of fast sodium currents obtained by the synchronous cumulation method (ten summations) are illustrated in Fig. 1a. The initial phase of development of the inward currents and the outward sodium currents can be seen at potentials above the reversal potential. Current—voltage characteristics of peak values of the sodium current are shown in Fig. 1b.

To determine how conductance of the sodium channels depends on potential, the momentary current—voltage characteristics were measured by the usual method [6]. The membrane was stimulated by conditioning pulses of the same magnitude. During the descending phase of the ionic current the membrane potential was reversed in steps to different levels (Fig. 2a). The strength of the current measured immediately after reversal of the potential was plotted as a function of the change of potential (Fig. 2b). Within the range of potentials tested (120 mV) the momentary current—voltage characteristic curve was linear. These observations agree with the conclusion drawn previously [4].

The time constants of activation (τ_m) were determined relative to the ascending phase of the current on the assumption that changes in sodium conductance are described by equations of the Hodgkin—Huxley type [7]:

$$g_{Na}(t) = \bar{g}_{Na} \cdot m^k(t) \cdot h(t),$$

where

$$g_{Na} = I_{Na}/(V - V_{Na});\ m(t) = m_\infty - (m_\infty - m_0)\, e^{-\frac{t}{\tau_m}}.$$

The ascending phase of the current after introduction of a correction for inactivation H(T) [1] was satisfactorily described in every case by the equation:

$$g^*_{Na}(t) = \frac{g_{Na}(t)}{h(t)} = \bar{g}_{Na} m^2(t), \text{ for } K=2.$$

The measured values of the time constant of activation (τ_m) are given in Fig. 3a. The curve of τ_m as a function of membrane potential was bell-shaped. Values of τ_m at potentials below the activation threshold were calculated by doubling the time constant of exponential decay of the current, by the procedure described for measurement of the momentary current–voltage characteristic curve. τ_m, estimated on outward sodium currents by the time to the peak of the current, by the equation:

$$t_p = \tau_m \ln \left[\left(\frac{m_\infty - m_0}{m_\infty} \right) \left(\frac{2\tau_h + \tau_m}{\tau_m} \right) \right],$$

changes from 40 to 30 μsec depending on potential.

Dependence of the steady-state level of activation (m_∞) on potential was measured by two methods. In the first method [6] the descending phase of the current was extrapolated [1] to the beginning of development of the current. The value obtained after division by ($V - V_{na}$) was directly proportional to m_∞. In the second method dependence of the peak conductance — the peak current divided by ($V - V_{Na}$) — on potential was determined (Fig. 3b). This value is described by the equation:

$$g_{Na} = \bar{g}_{Na} m_\infty^2 \cdot h_0 \left(\frac{2\tau_h}{\tau_m + 2\tau_h} \right)^2 \left(\frac{m_\infty}{m_\infty - m_0} \cdot \frac{\tau_m}{\tau_m + 2\tau_h} \right)^{\frac{\tau_m}{\tau_h}}.$$

Given that $2\tau_h \gg \tau_m$, the value of $\sqrt{g_{Na}^{peak}/\bar{g}_{Na} \cdot h_0}$ gives the approximate value of $m_\infty(V)$. The dependence of $m_\infty(V)$, measured by the two methods, is illustrated in Fig. 3c.

Time resolution attained by the patch voltage clamp method enabled the outward sodium currents to be recorded at potentials above the reversal potential and the ascending phase of the inward sodium currents to be reliably distinguished. The experimental description of activation of the fast sodium current is given to correspond to the Hodgkin–Huxley model as the most generally accepted method of presenting experimental results.

The authors are grateful to Z. A. Bendukidze, N. A. Burnashev, and B. Shubert for great help at all stages of the work and to B. I. Khodorov for useful discussion of the results.

LITERATURE CITED

1. Yu. I. Zil'berter and E. N. Timin, Byull. Éksp. Biol. Med., No. 10, 5 (1982).
2. Yu. I. Zil'berter, E. N. Timin, Z. A. Bendukidze, et al., Byull. Éksp. Biol. Med., No. 12, 759 (1981).
3. A. N. Brown, K. S. Lee, and T. Powell, J. Physiol. (London), 318, 455 (1981).
4. A. M. Brown, K. S. Lee, and T. Powell, J. Physiol. (London), 318, 479 (1981).
5. L. Ebihara and E. A. Johnson, Biophys., J., 32, 779 (1980).
6. A. L. Hodgkin and A. F. Huxley, J. Physiol. (London), 116, 473 (1952).
7. A. L. Hodgkin and A. F. Huxley, J. Physiol. (London), 117, 500 (1952).
8. T. Powell, D. A. Terrar, and V. W. Twist, J. Physiol. (London), 302, 131 (1980).