to the second stimulus. Values of the parameter $\overline{m}$ in these experiments were found to be considerably greater than 1. Consequently, activity of several release points was recorded.

At the same time, it was found that the inequalities $N(11/1) > \overline{p}_1$ and $N(111/11) > \overline{p}_1$ were satisfied, where $\overline{p}_1$ stands for the probability of quantum transmitter release to the first stimulus. This is evidence that the number of Ca^{++} ions leaving the cytoplasm of the nerve ending in response to secretion of a portion to acetylcholine is smaller than the quantity of calcium entering the terminal during depolarization of the presynaptic membrane. Hence it follows that, despite the continuous loss of calcium as a result of transmitter secretion, during repetitive stimulation the intracellular calcium concentration will rise.

The presence of two mechanisms reducing the calcium concentration in a nerve ending can thus be postulated. The first mechanism is independent of transmitter secretion and is due to the functioning of intracellular structures which utilize Ca^{++} ions from the axoplasm of the nerve ending (mitochondria, endoplasmic reticulum, etc.) [3]. The second mechanism is directly connected with quantum transmitter secretion or with processes lying at its basis.

LITERATURE CITED

1. A. L. Zefirov and E. L. Stolov, Neirofiziologiya, 14, 326 (1982).
2. M. A. Kamenskaya, Usp. Fiziol. Nauk, 3, 23 (1972).
3. M. P. Blaustein, R. W. Ratzlaff, and N. K. Kendrick, Ann. N. Y. Acad. Sci., 307, 195 (1978).
4. E. W. Johnson and A. Wernig, J. Physiol. (London), 218, 757 (1971).
5. B. Katz and R. Miledi, J. Physiol. (London), 189, 535 (1967).
6. A. Mallart and A. R. Martin, J. Physiol. (London), 193, 679 (1967).
7. A. Wernig, J. Physiol. (London), 244, 207 (1975).
8. R. S. Zucker, J. Physiol. (London), 229, 787 (1973).

INACTIVATION OF THE FAST SODIUM CURRENT ACROSS THE MEMBRANE OF SINGLE HEART CELLS

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Many investigations have been devoted to the study of inactivation of sodium channels in electrically excitable membranes. Recent investigations [2, 3, 7, 9] have revealed deviations in the development of inactivation from the monoexponential course predicted by the Hodgkin–Huxley model [6]. Contradictory information on this subject has been obtained by experiments on single heart cells [2, 4].

In the present investigation inactivation of the sodium current was studied in the membrane of single heart cells by the microrecording technique described in [1]. The preliminary results were described previously.

EXPERIMENTAL METHOD

Cells were isolated by the method in [8]. The experimental procedure was described previously. A cell selected under the microscope was transferred to a working chamber containing solution of the following composition (in mM): NaCl – 130, KCl – 5.4, $MgSO_4$ – 1.2, $CaCl_2$ – 0.9, glucose – 11, MOPS buffer – 20, pH 7.4. The experiments were carried out at room temperature (20-22°C). A V-shaped polyethylene suction cap, with a pore 5-7 μ in diameter, was applied to a small area of cell membrane. The solution in the cap had the composition described above, with the addition of 1 mM $MnCl_2$ and 1 mM 4-aminopyridine to block the calcium and potassium channels respectively.

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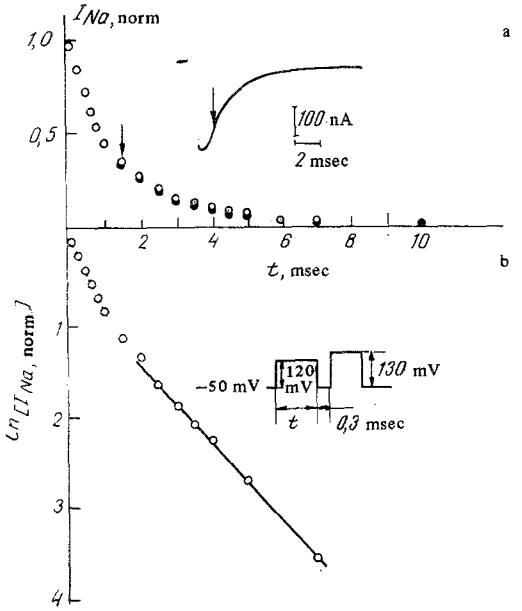

Fig. 1. Time course of inactivation of sodium conductance at a potential of 70 mV. a) Empty circles – results of measurement by two-pulse method. Currents normalized for response to test pulse without conditioning pulse; filled circles – descending phase of sodium current in conventional units, on same scale as results of measurements by two-pulse method. Inset: sodium-current recorded at a potential of 70 mV. b) Time course of inactivation (two-pulse method) plotted between semilogarithmic coordinates. Inset: pulse program.

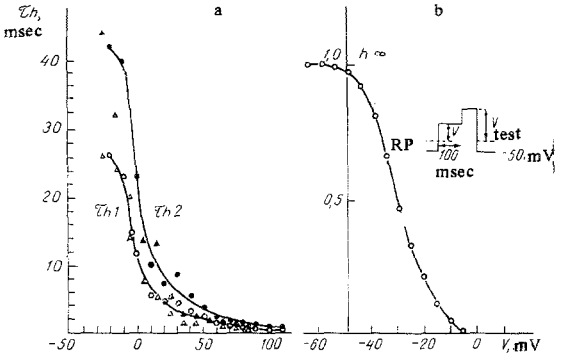

Fig. 2. Dependence of time constants τ_{h1} and τ_{h2} (a) and steady-state level (b) of inactivation on potential. a) Different symbols correspond to different experiments: empty symbols – τ_{h1}, filled symbols – τ_{h2}. Continuous curves traced visually. b) Inset: program of pulses. Amplitude of testing potential V_{test} = 70 mV.

Currents were measured by means of a "virtual ground" circuit with subsequent filtration to the 5 kHz band and recording on a VC-9 oscilloscope (Nihon Kohden, Japan) or ATAC-250 digital averager (from the same firm). Potentials were assigned from the level of the resting potential.

EXPERIMENTAL RESULTS

The kinetics of the inactivation process were studied both by the two-pulse method [5] and also directly from the time course of the descending phase of the sodium current. In the two-pulse method (Fig. 1b, inset) a conditioning pulse, whose duration varied, was followed by a standard test pulse. Dependence of the amplitude

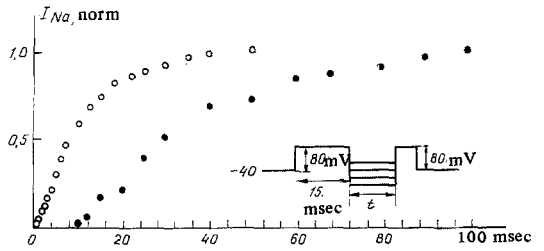

Fig. 3. Recovery of sodium conductance from inactivation at potentials of −30 mV (filled circles) and −80 mV (empty circles). Inset: program of pulses. Currents normalized for response to test pulse, following conditioning pulse after an interval of 200 msec.

of the response to the test pulse on the duration of the conditioning pulse was studied. This relationship is shown in Fig. 1a (empty circles) for a conditioning pulse with an amplitude of 70 mV. The descending phase of the sodium current (filled circles), recorded at the same potential, is also shown in Fig. 1a (inset). The time course of inactivation, plotted between semilogarithmic coordinates, is shown in Fig. 1b. At least two exponential curves can be seen to be present. The same picture was observed over a wide range of potentials studied. Here and later, only two exponential curves have been distinguished. The time constants of the two exponential functions are potential-dependent. The results of measurements by the two-pulse method and by direct logarithmic plotting of the descending phase were approximately the same.

Dependence of the time constants τ_{h1} and τ_{h2} on potential, measured by the two-pulse method, are shown in Fig. 2a. The values of τ_{h1} and τ_{h2} vary depending on potential from 27 to 0.3 msec and from 42 to 0.6 msec.

Dependence of the steady-state level of inactivation h_∞ on potential (Fig. 2b) was measured by the two-pulse method [5]. Testing pulses of constant voltage were preceded by 100-msec conditioning pulses of different amplitude.

To record the recovery of sodium conductance, the sodium current was inactivated by a conditioning pulse. The amplitude of the current in response to the testing pulse, following the conditioning pulse after different time intervals, was measured. The recovery process was recorded at potentials of −30, −40, −50, −60, −70, and −80 mV. Curves showing emergence of sodium channels from inactivation at potentials of −30 and −80 mV are illustrated in Fig. 3. The curves are S-shaped, with a marked delay at the beginning. The amount of delay was determined formally by Chiu's procedure [3]. The length of the delay varied depending on the potentials from 11 to 0.5 msec, reducing with shifts of potential toward hyperpolarization. The results were not subjected to theoretical analysis.

The results thus show that the kinetics of inactivation of sodium conductance in the heart cell membrane does not correspond to the Hodgkin–Huxley model. These results agree qualitatively with those obtained previously on the Ranvier node membrane [3] and single heart cells [2].

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LITERATURE CITED

1. Yu. I. Zil'berter, E. N. Timin, Z. A. Bendukidze, et al., Byull. Éksp. Biol. Med., No. 12, 759 (1981).
2. A. M. Brown, K. S. Lee, and T. Powell, J. Physiol (London), 318, 479 (1981).
3. S. Y. Chiu, J. Physiol. (London), 273, 573 (1977).
4. L. Ebihara, N. Shigeto, M. Lieberman, et al., J. Gen. Physiol., 75, 437 (1980).
5. A. L. Hodgkin and A. F. Huxley, J. Physiol. (London), 116, 497 (1952).
6. A. L. Hodgkin and A. F. Huxley, J. Physiol. (London), 117, 500 (1952).
7. W. Nonner, J. Physiol. (London), 299, 573 (1980).
8. T. Powell, D. A. Terrar, V. W. Twist, et al., J. Physiol. (London), 302, 131 (1980).
9. C. L. Schauf, Biophys. J., 14, 151 (1974).