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Characterization of the Inactivation Process of the Sodium Channel in Frog Ventricular Cells

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ABSTRACT

The voltage clamp experiments were conducted on single frog ventricular cells with the oil gap method for characterization of the sodium current (I_{Na}). The results obtained can be summarized as follows:

1. The falling phase of I_{Na} could be fitted by a single-exponential function when the series resistance was small (0.81—2.25 MΩ) and compensated.
2. Normalized steady-state inactivation ($h\infty$) curve could be fitted by the equation $h\infty = 1/(1+\exp(V-V_h)/k)$, where the half-inactivation voltage (V_h) was -59.8 mV and the slope factor (k) was 5.64 mV. No shift of this curve was observed throughout the experiment.
3. There was a delay of onset of inactivation development.
4. The time course for recovery of I_{Na} from inactivation exhibited a single time constant.
5. Almost all properties of the inactivation process of Na channel can be described by the original Hodgkin-Huxley's (H—H) kinetic model, except the presence of the delayed onset of inactivation. In order to incorporate this discrepancy, modification of H—H model is required, that the transition rate constant from the open state to the inactivated state may well be larger than that from the closed state to the inactivated state.

Key words: *Na-current, Voltage-clamp method, Inactivation, Single frog ventricular cell*

Since sodium current (I_{Na}) is a main component of the generation of excitation in the heart, many investigations have been made by using various methods[13]. There have been several reports based on the data obtained by the voltage clamp that the general characteristics of I_{Na}, such as configuration of I—V curve and selectivity of ion, in the cardiac muscle are similar to those in the nerve cell[2,4-8,15,16,19,23]. However, noticeable difference in the characters of I_{Na} in the mammalian ventricular cell[6,15,23] has been reported that the falling phase of I_{Na} consists of several components each having a different time constant. When the mammalian ventricular cell is used in the voltage clamp experiment, transverse tubular (T-tube) system gives rise to two obstacles, that is, enlargement of the surface area and accumulation of ions[22,26]. Histologically, frog ventricular cells are preparations more suitable to the voltage clamp, because of the lack of T-tube[26].

Seyama and Yamaoka[25] have reported that the falling phase of I_{Na} in the frog ventricular cell is governed by a single exponential function. Controversy in the number of the components of the falling phase of I_{Na} among various cardiac cells may well be due to the difficulty in the control of membrane potential under the voltage clamp.

Since in the oil gap method the area of the cell membrane under control is reduced to less than half of the cell and the series resistance between the cell membrane and the intracellular electrode is much smaller than that in the suction pipette method, more favorable voltage clamp condition can be obtained[20]. Thus, it is worthwhile to reinvestigate the time course of I_{Na} with this method, using frog ventricular cells. Special attention has been paid to determine the time course of inactivation process of I_{Na} in order to construct the kinetic model.

MATERIALS AND METHODS

Preparations

The method employed for making single cell preparation of the frog, *Rana Catesbeiana*, is essentially the same as previously described[25]. The composition of the Ca-free Ringer solution was so-

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dium aspartate 113.5, $MgSO_4$ 3.0, CsOH 5.4, HEPES 5.0, aspartate 5.4 (in mM). pH was adjusted to 7.2.

In order to eliminate calcium current, Mg^{++} is substituted for Ca^{++}, because Mg^{++} is much less permeable through Ca channel and has a similar stabilizing effect[11,14].

Internal solution consists of CsOH 130.0, Na_2ATP 5.0, creatinine phosphate (sodium salt) 5.0, $MgSO_4$ 5.0, glucose 10.0, EGTA 5.0, HEPES 10.0, and aspartate 100.0 (in mM). pH was adjusted to 7.0. Temperature during the experiment was room temperature of about 18°C.

Electrical recording

The method used in this experiment which was originally developed by Mitsuiye and Noma (1987)[20] is particularly beneficial to overcome the difficulty of the voltage clamp, because (1) the resistance access to the cell membrane from the intracellular electrode is made smaller than that in the suction pipette method by tearing off the end of the cell in the intracellular section and (2) the area where the excitation occurs is substantially reduced by placing less than half of a cell in the extracellular section.

The procedure for estimating the series resistance (Rs) consisting of resistance of the electrode and that of cytoplasm is as follows: (1) membrane capacitance (C) is measured by estimating i_m in the application of ramp pulse having a constant rate of rise (5.0 V/sec). When the change in membrane potential is well below the threshold for I_{Na}, $i_m = C \cdot dV/dt$. C is estimated to be 13.2—78.3 pF. (2) Because membrane resistance (r_m) is much greater than Rs, the time constant (τ) for decaying capacitive current in response to small depolarizing pulse is given by equation $\tau = Rs \cdot C$. τ is calculated to be in the range between 31 and 116 μs and consequently Rs in the range between 0.81 and 2.25 MΩ.

Adoption of single exponential function to the falling phase of I_{Na} was quite successful, when Rs's were in the range described above (Fig. 1, a).

When the cell membrane was torn incompletely, the series resistances were measured to be beyond 3 MΩ and good voltage clamp could not be achieved. In these cases, the falling phase of I_{Na} could not be fitted by a single exponential function (Fig. 1, b).

Since the specific membrane capacitance of frog ventricular cell is 1 $\mu F/cm^2$ [25], the area of the cells controlled by the voltage clamp experiment was in the range between 1320 and 7830 $\times 10^{-8} cm^2$.

When recorded data were fitted to the equations, the Marquardt algorithm[18] was used to minimize the least square error between the data and the equation.

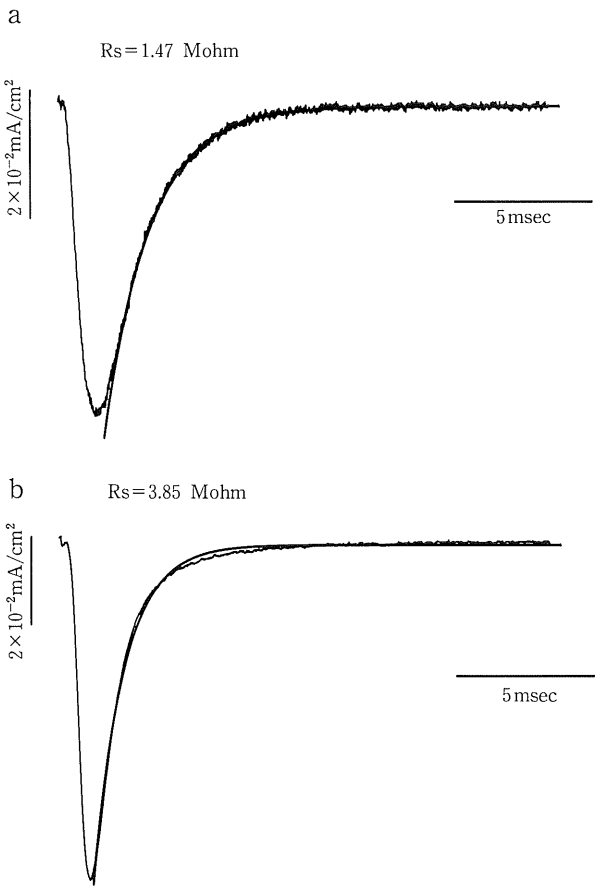

Fig. 1. Fitting of single exponential curve to the falling phase of I_{Na}. Current trace (a,b): I_{Na} in response to cathodal pulse of −20 mV from a holding potential of −100 mV. Fitting curves (a,b): curve fitted by equation $A \times \exp(-t/\tau) + B$. Series resistance a: 1.47 MΩ b: 3.85 MΩ.

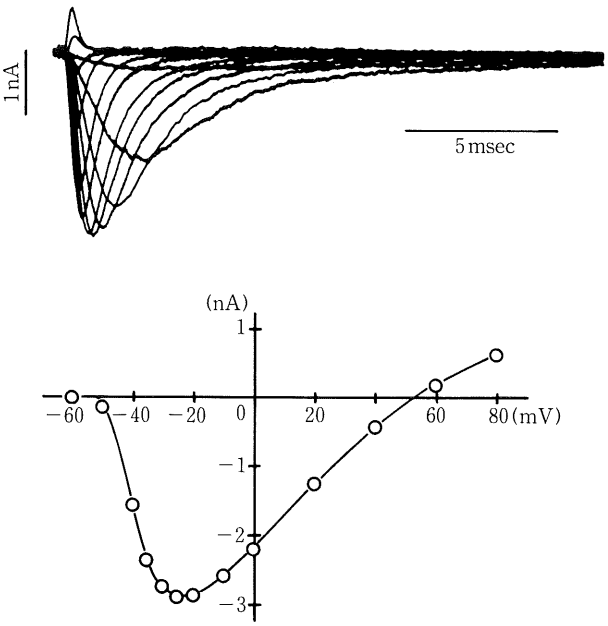

Fig. 2. Current—voltage relationship. Inset figure: a family of I_{Na} traces in response to cathodal pulses from a holding potential of −100 mV. Graph: the peak I_{Na} against the membrane potentials.

RESULTS

I—V curve

The membrane was held at −100 mV and was stepped to various membrane potentials. This elicited the transient current which decayed to the base line in a single exponential manner.

I—V curve crosses the voltage axis at 54 mV, which is very close to the value calculated by the Nernst equation for sodium ion. Threshold for I_{Na} appears to be around −50 mV (Fig. 2).

Steady-state inactivation curve

The steady state inactivation curve was experimentally determined by giving 500 msec conditioning pulses of various amplitudes followed by a standard test pulse to 0 mV. The empirical equation $h\infty = 1/(1+\exp(V-V_h)/k)$[12] were adopted to the data, having a membrane potential (V_h) of −59.8 mV at which half inactivation occurs and a slope factor (k) of 5.64 mV (Fig. 3).

According to the several reports, the steady-state inactivation curve during the whole cell voltage clamp is shifted to hyperpolarization by 20 mV in the mammalian cardiac cells[8-10,15,17,24]. It is worthwhile to test whether or not a similar shift of the inactivation curve occurs in the frog ventricular cell. As shown in Fig. 3, the steady state inactivation curves obtained at 1, 6 and 16 min after the experiment was commenced remained constant, indicating that there was no shift in the inactivation curve.

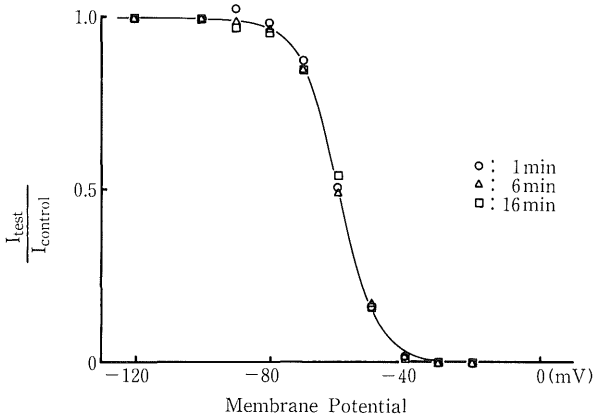

Fig. 3. Normalized steady-state inactivation (h∞) curve with 500 msec conditioning pulse. h∞-curves in solid lines recorded at 1, 6 and 16 min after the experiments were started did not shift along the membrane potential axis.

Development of inactivation of I_{Na}

The development of inactivation of I_{Na} was obtained with the double pulse method, in which conditioning pulses of various durations were applied prior to a standard test pulse to 0 mV. In order to achieve deactivation of the gate activated by the conditioning pulse, a 1 msec-gap was set between the conditioning pulse and the test pulse. In order to minimize the effect of series resistance on the time course of I_{Na}, experiments in this series were carried out in the medium containing 25%

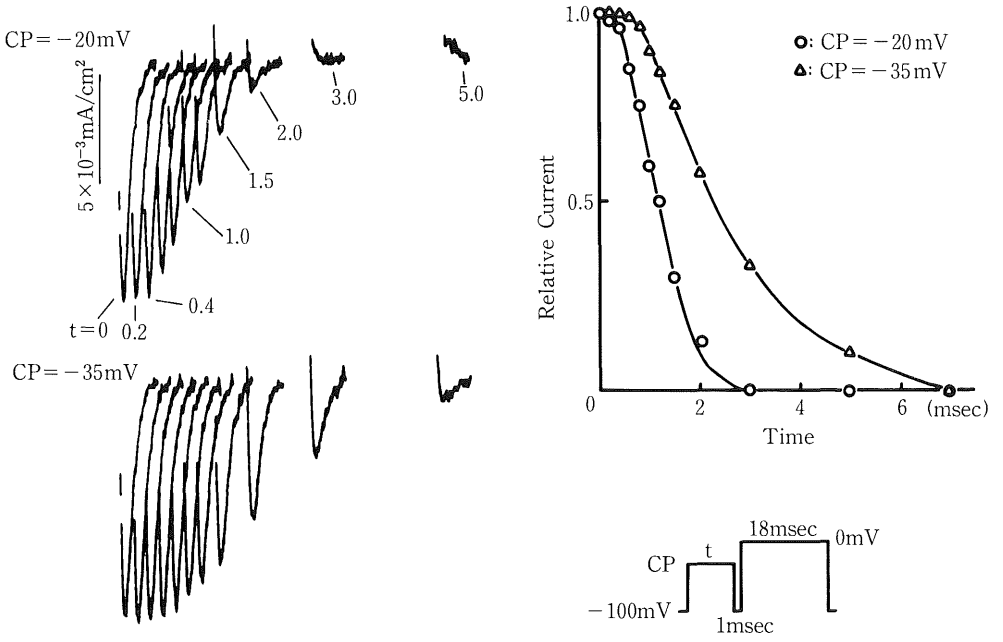

Fig. 4. The onset of inactivation development demonstrated with a two-pulse procedure. Inset figures: Current trances for conditioning pulse (CP) of −20 mV (upper) and that of −35 mV (lower) during the second pulse. Numerals in upper inset figure showing the duration of the conditioning pulses are also applicable to lower inset figure. Holding potential was −100 mV in 25% Na^+ (28.4mM Na^+) external solution. Graph gives the time course of the inactivation. Ordinate: peak I_{Na} during second pulse expressed as a fraction of I_{Na} without conditioning pulse (t=0). Abscissa: duration of conditioning pulse (t).

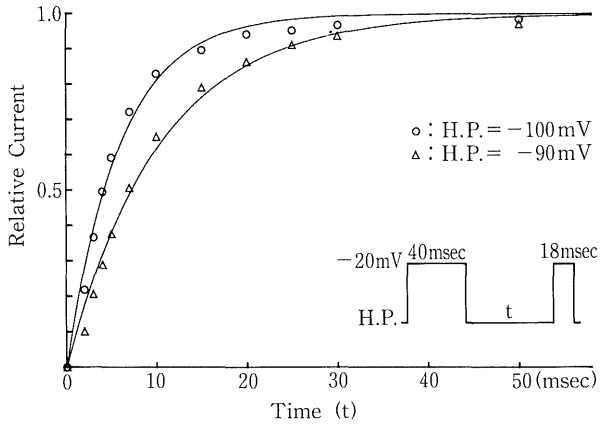

Fig. 5. Recovery from inactivation. ○ : Holding potential (H.P.)=−100mV △ : H.P.=−90mV Ordinate: peak I_{Na} during second pulse expressed as a fraction of I_{Na} without conditioning pulse. Abscissa: interval between the end of first pulse and beginning of second pulse. Fitting curves: curve fitted by the equation $1-\exp(-t/\tau)$; τ =6.02 in H.P.=−100mV, τ = 10.42 in H.P.=−90mV.

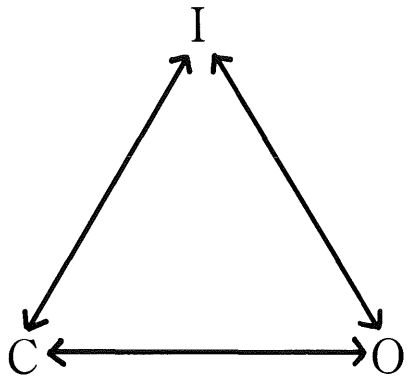

Fig. 6. The kinetic model of a sodium channel. C: closed state which may consist of several states. O: open state. I: inactivated state.

$[Na]_o$(28.4 mM). The delay of onset of the inactivation development was clearly demonstrated in both conditioning pulses of -20 and -35 mV (Fig. 4). This finding does not reconcile with the H—H equation[12].

Recovery from inactivation

Inactivation gate which had been closed at the end of long depolarization gradually opened when the membrane was reporalized. Time course of recovery from closed state was measured by sending a pair of depolarizing pulses. The first pulse of 40 msec is long enough to induce complete inactivation and the second pulse is given to estimate the amount of Na conductance available after the priming intervals of various durations. Single exponential curves made a good fit to these original data (Fig. 5).

DISCUSSION

The series resistance and the effective capacitance in this method, which are critically important for good spatial control of the membrane potential, were estimated to be 0.81—2.25 MΩ and 13.2—78.3 pF, respectively. While in the whole cell clamp mode these corresponding parameters in the frog ventricular cells have been reported to be 6.03—6.55 MΩ and 48.1—56.3 pF[25]. Reduction in these values provides substantial improvement in the quality of the membrane potential control under the voltage clamp condition. It has been reported that the rise time from 10 to 90% of the step pulse of the membrane potential is less than about 100 μ sec in the oil gap method (personal communication, Mitsuiye and Noma), which is more than 10 times faster than that in the previous experiment[25]. In the improved condition h_∞-curve did not shift throughout the experiment, and both the falling phase of and the recovery from inactivation of I_{Na} are governed by a single exponential function. Moreover, there is a delay of onset of inactivation development. The previous report[25] has shown that the time course of the development of and the recovery from the inactivation of sodium channel as well as the falling phase of I_{Na} are in single exponential function. Thus, when compared with the previous report[25], sole difference in the data on the character of I_{Na} is the presence of delay of onset of inactivation. Original H—H kinetic model[12] requires all the parameters for inactivation process to be single exponential function. In order to incorporate these new findings into the kinetic model, it is proposed that the rate constants for inactivation state are different; the transition rate constant from O to I may well be larger than that from C to I (Fig. 6). This model in the extreme condition becomes identical to that proposed by Armstrong & Bezanilla[1,3] in squid giant axon; channels must open before they can be inactivated[21]. At present, it is difficult to decide how many distinct closed states are involved in this kinetic scheme.

It has been reported that the falling phase of I_{Na} consists of two components instead of one in the mammalian cardiac cell. The difference may well be caused by ion accumulation or depletion in T-tube or incomplete spatial control of the membrane potential. Another possible reason is the species difference in the character of the Na channel, such as reopening of the once inactivated Na channel[15].

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