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An Investigation on the Inactivation Process of Sodium Currents in Single Frog Ventricular Cells

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Abstract The characteristics of sodium currents (I_{Na}) in single frog ventricular cells were studied with the oil gap method. This method improves time- and space-control of the membrane potential under the voltage clamp, thereby making possible accurate analysis of fast events of I_{Na}. In this preparation the threshold of I_{Na} was about -60 mV and the reversal potential was 58 mV, which is close to the value calculated by the Nernst equation for sodium ions. Because the instantaneous current-voltage (*I-V*) relationship is linear, the ease of permeation of sodium ions through Na^+ channels is well expressed by the chord conductance. The falling phase of I_{Na} and the time course of recovery from inactivation follow a time course of single exponential function. The time constants for on- and off-processes of inactivation at the same membrane potential are very close to each other, indicating only a single state of inactivation. Though almost all properties of I_{Na} were well described by Hodgkin-Huxley's model, a clear delay of onset of inactivation was demonstrated by two-pulse experiment. In this report the modified kinetics scheme was proposed which can account for both a delay of onset of inactivation development and a falling phase of I_{Na} that follows a single exponential time course.

Key words: sodium current, inactivation, single frog ventricular cell, voltage clamp method.

Methods developed recently for isolation and spatial control of the membrane potential of single myocardial cells has made it possible to analyze fast I_{Na} quantitatively. There have been a number of reports using the voltage clamp method which indicate that the general properties of the cardiac Na^+ channel are similar to those in nerve cells (BROWN *et al.*, 1981a, b; BODEWEI *et al.*, 1982; BUSTAMANTE and MCDONALD, 1983; CACHELIN *et al.*, 1983; MATSUKI and HERMSMEYER, 1983; BENNDORF *et al.*, 1985; KUNZE *et al.*, 1985; PATLAK and ORTIZ, 1985). However, observations by some investigators of subtle differences in the time course of

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inactivation of I_{Na} in mammalian heart cells suggest that both development of and recovery from inactivation behave in a multi-exponential manner (BROWN *et al.*, 1981b; KUNZE *et al.*, 1985; PATLAK and ORTIZ, 1985). On the contrary, SEYAMA and YAMAOKA (1988) have shown that Hodgkin-Huxley (H-H) formalism (HODGKIN and HUXLEY, 1952) is applicable to the Na^+ channel of frog ventricular cells, indicating that all these processes of inactivation could be expressed by a single exponential function. Since their whole cell clamp method could not drive the membrane to a new potential within 1 ms, during which time the crucial phenomena to discriminate the model, such as delay of onset of inactivation, may occur, they were only able to obtain suggestive but not conclusive evidence that inactivation occurs in a single exponential manner without delay. In order to clarify the process of inactivation, it is essential to use a much faster voltage clamp method. The oil gap method recently developed by MITSUIYE and NOMA (1987) retains electrical characteristics suitable for this experimental purpose, because the area of the voltage-clamped membrane and the series resistance between the intracellular electrode and effective cell membrane, which are two main factors for inducing the slow rising time of the pulse in the voltage clamp experiment, could be substantially reduced. An attempt was therefore made to reinvestigate the properties of I_{Na} of the frog ventricular cell under these more favorable voltage clamp conditions, with particular reference to the time course of inactivation in the on- and off-process.

MATERIALS AND METHODS

Preparation and solutions. The method employed for enzymatically dispersing single ventricular cells of the frog, *Rana catesbeiana*, was essentially the same as that by SEYAMA and YAMAOKA (1988).

The compositions of all the solutions in these experiments are given in Table

Table 1. Composition of solutions (mM).

	External solution (E_1)	25% Na external solution (E_2)	Internal solution (I)
Sodium aspartate	113.5	28.4	—
Na_2ATP	—	—	5.0
Sodium creatine phosphate	—	—	5.0
CsOH	5.4	90.5	130.0
$MgSO_4$	3.0	3.0	5.0
Glucose	—	—	10.0
EGTA	—	—	5.0
HEPES	5.0	5.0	10.0
Aspartic acid	5.0	118.9	100.0
pH	7.2	7.2	7.0

1. In almost all the experiments, E_1 was used as the external solution and I as the internal solution. Ca^{2+} was replaced by Mg^{2+} in order to eliminate calcium currents, as Mg^{2+} is much less permeable through Ca^{2+} channels (HESS *et al.*, 1986) and has a similar stabilizing effect (FRANKENHAEUSER and HODGKIN, 1957). Cs^+ was substituted for K^+ because Cs^+ blocks only outward currents through K^+ channels (ADELMAN and SENFT, 1968), and Cl^- was substituted for aspartate. Albumin (0.25%) was added to the external solution in order to prevent spontaneous rupture of the cell membrane by reducing direct contact between the cell membrane and the oil (MITSUIYE and NOMA, 1987).

Electrical recording. The method for setting up the single cell preparation used in this experiment was essentially the same as the oil gap method developed by MITSUIYE and NOMA (1987). Briefly, the principle of this method is the same as that of the single sucrose gap method. Paraffin oil is used as an electrical insulator instead of sucrose solution. An isolated cell was placed in the compartment of the external solution and then its end was sucked with a fine glass-microelectrode attached to a manipulator. Two-thirds of the cell was pulled out of the external solution into the oil compartment. Another large glass pipette filled with the internal solution and having a tip diameter of about 0.3 mm, which was mounted on another manipulator, was slowly lifted up so that a part of the cell in the oil compartment could dip into the internal solution. Finally, a part of the cell in the internal solution compartment was destroyed by crushing the tip of a fine microelectrode against the cell on the bottom of the large glass pipette.

The procedure for estimating the series resistance (R_s) was as follows. (1) Membrane capacitance (C) of the effective cell membrane is measured by estimating i_m when applying a ramp pulse ($dV/dt = 5.0$ V/s). When the change of membrane potential is well below the threshold for I_{Na}, $i_m = C \cdot dV/dt$. C was estimated to be 31.2 ± 11.6 pF ($n = 35$). (2) Because the effective membrane resistance is much greater than R_s, the time constant (τ_m) for decaying capacitive current in response to a small depolarizing pulse is given by the equation $\tau_m = R_s \cdot C$. τ_m was calculated to be 49.2 ± 18.2 μs ($n = 30$) and R_s to be 1.59 ± 0.40 MΩ ($n = 30$). Since the specific membrane capacitance of the frog ventricular cell can reasonably be assumed to be 1 μF/cm^2, the area of the effective cell membrane is in the range of $3.12 \pm 1.16 \times 10^{-5}$ cm^2 ($n = 35$). In the suction pipette method, R_s and C have been reported to be 6.29 ± 0.26 MΩ and 52.2 ± 0.41 pF, respectively (SEYAMA and YAMAOKA, 1988). Thus, the advantage of the oil gap method is a lower membrane capacitance (due to a reduction in the controlled area of the membrane), together with a lower series resistance resulting in a value of τ_m, which is 1/3 to 1/10 that of the suction pipette method. Since τ_m represents the time needed to charge the membrane under voltage clamp, the response of the membrane potential to follow quick changes in behavior of the Na^+ channel should be substantially improved. The resistance combined of the seal and the membrane effective resistance was estimated to be 416.4 ± 295.1 MΩ ($n = 22$) by measuring the leak current induced by a small depolarizing pulse. Since this value is much larger than the combined

resistance in the range between 6 and 20 MΩ measured in the guinea pig ventricular cell by MITSUIYE and NOMA (1987), it seems that the seal of oil compartment is effective enough to prevent internal solution from mixing with external solution.

When the activation of I_{Na} showed a noticeable delay or when the amplitude of I_{Na} near the threshold potential suddenly became large and unstable, the measured R_s always reached a value greater than 3 MΩ. These typical signs of poor spatial clamp of the membrane potential appear to be due to incomplete perforation of the cell membrane in the internal solution compartment. All the successful experiments were conducted after confirming the absence of these signs. The values of R_s measured afterward were always below 2 MΩ. The temperature throughout the experiments was about 18°C, except for the study on the instantaneous *I-V* relationship, which was carried out at 12°C.

Data acquisition and analysis. The pulses were generated by a 16-bit digital-to-analog converter (DAC-98, Canopus Denshi Co. Ltd., Kobe, Japan) connected to a personal computer (PC-9801, NEC., Tokyo, Japan) and were transferred to a voltage clamp amplifier (CEZ-2200, Nihon Kohden Co. Ltd., Tokyo, Japan). At the same time, records from the amplifier were digitized with a 12-bit analog-to-digital converter (ADX-98, Canopus Denshi Co. Ltd., Kobe, Japan) using a sampling interval of 20 μs and then transferred to the same computer for storage on a floppy disc. The stored data were further analyzed on the computer.

The recorded data were fitted to the equations by Marquardt algorithm (MARQUARDT, 1963) to minimize the least square error between the data and the equation. Data are presented as mean ± S.D. (number of observations), unless otherwise stated.

RESULTS

I-V relationship

The membrane was held at −100 mV and was subjected to 18 ms test pulses in 10 mV increments. This elicited transient inward currents. All the records of I_{Na} were obtained by subtracting background membrane currents from those in response to the test pulses. As for the procedure to obtain the background current, after I_{Na} was completely inactivated by a conditioning pulse of −20 mV for 500 ms prior to the test pulse, the remaining membrane current during the test pulse was recorded as background current. The falling phase of I_{Na} consists of a single exponential component. The *I-V* curve crosses the voltage axis at around 58 mV, which is very close to the value calculated by the Nernst equation for sodium ions. The threshold for I_{Na} is around −60 mV and the maximum amplitude of I_{Na} is observed at around −30 mV.

The double-pulse method was employed to analyze the fully activated *I-V* relationship. The first pulse depolarized the membrane from the holding potential of −100 to 0 mV for a duration of 1.8 ms, followed by a second pulse of variable

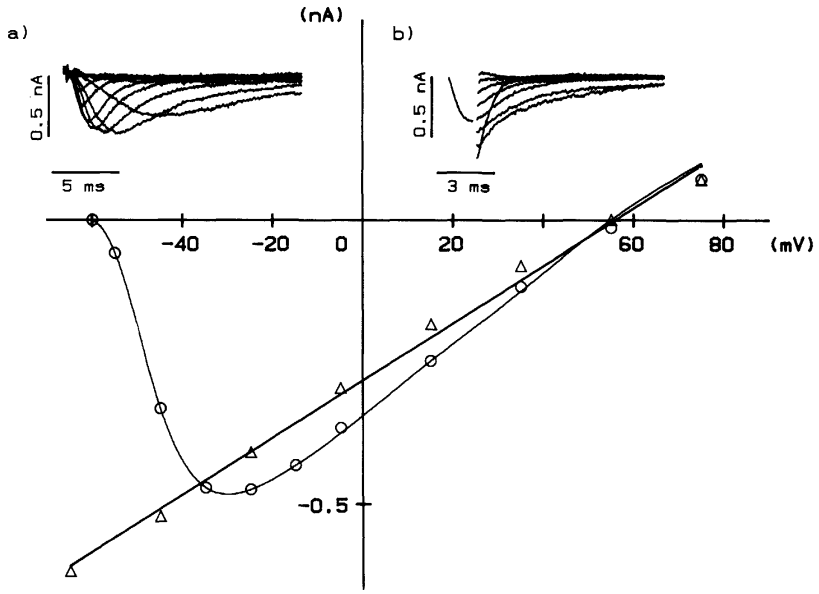


Fig. 1. Conventional and instantaneous *I-V* relation. Inset a) indicates a family of I_{Na} traces and b) a family of tail currents. Graph shows the conventional *I-V* relation (open circles) and the instantaneous *I-V* relation (open triangles). Ordinate indicates the amplitude of peak I_{Na} in the former case and that of the tail current immediately after 0.24 ms blanking-out period in the latter case. Abscissa indicates the membrane potential of the pulse for the former and that of the second pulse for the latter.

amplitudes which changed the membrane potential to between −65 and 75 mV. Because the voltage clamp amplifier has to supply additional current on top of the peak I_{Na} through the series resistance, it takes more time to charge the membrane in response to the second pulse. Thus, the data for the first 0.24 ms of the second pulse were blanked out. To increase the time resolution of I_{Na}, the temperature at which this experiment was conducted was reduced to 12°C. Since, at this temperature the time course of the inactivation process of I_{Na} is relatively slow compared with the rising time of the second pulse, serious errors due to the blanking interval will not occur. Currents immediately after the blanking interval were taken as instantaneous current. Figure 1 shows that the instantaneous *I-V* relationship is linear and the ease with which sodium ions pass through the membrane is well described by the chord conductance.

Steady-state inactivation curve

The steady-state inactivation (h_∞) curve (HODGKIN and HUXLEY, 1952) was determined by a standard test pulse to 0 mV with a 500 ms conditioning pre-pulse of variable amplitudes. It has been reported that experiment with either the whole cell or single channel recording method on mammalian heart cells reveal a shift of

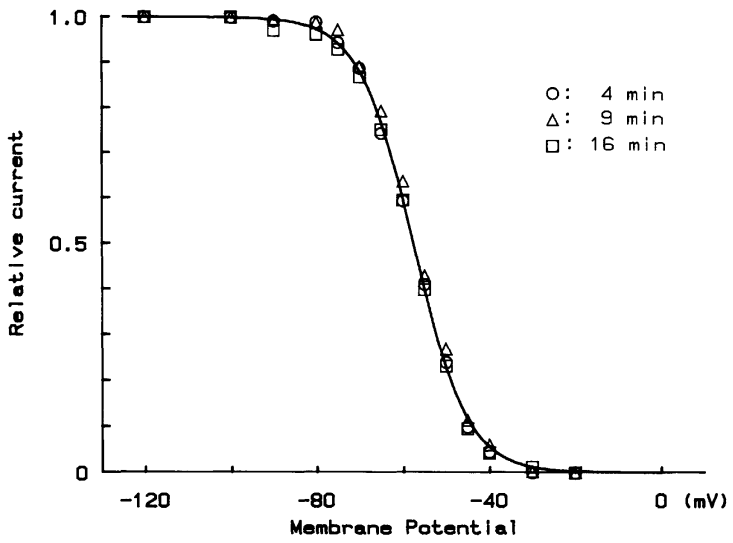


Fig. 2. h_∞-curve. The curve was well fitted by the empirical equation $h_\infty = 1/(1+\exp(V-V_h)/k)$, having a half inactivation voltage (V_h) of -60.0 ± 2.0 mV ($n=14$) and a slope factor (k) of 6.25 ± 0.52 mV ($n=14$). Different symbols indicate the data obtained at times elapsed (shown in numerals) after the experiment was commenced.

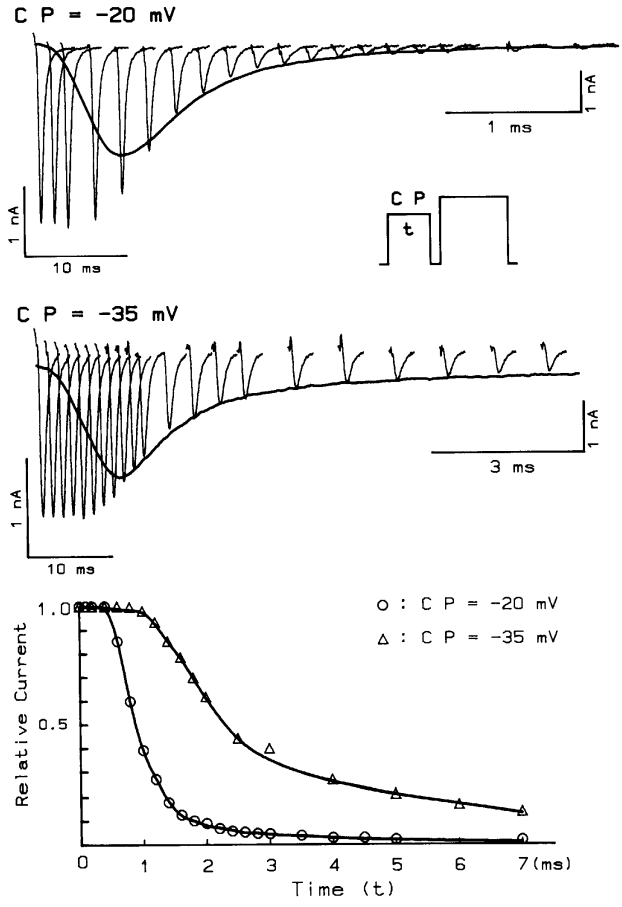
h_∞-curve in the hyperpolarizing direction of 25–50 mV within 15–80 min after the start of the experiment (CACHELIN *et al.*, 1983; KUNZE *et al.*, 1985; MAKIELSKI *et al.*, 1987). In this experiment, special attention was paid to this curious phenomenon

Fig. 3. Development of inactivation. Upper two panels show the composite pictures which consist of the peak inward currents with the double-pulse method (thin line) (scale in the left-hand corner) and the conventional I_{Na} at the same membrane potential of CP (thick line) (scale in the right-hand corner). The peak inward currents are obtained by the second pulses to 0 mV following conditioning pulses of various durations {those membrane potentials (CP) are shown in the upper left-hand corner in each panel}. The duration (t) of each conditioning pulse was 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.5, and 4.0 ms in CP $=-20$ mV and was 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ms in CP $=-35$ mV. The way in which the conventional I_{Na}'s are superimposed on the groups of peak inward currents is as follows: 1) in the case of CP $=-20$ mV, the steady state current of the conventional I_{Na} was set to be zero; 2) in CP $=-35$ mV, since h_∞-curve does not become zero (see Fig. 2), the trace of the conventional I_{Na} was shifted downward so as to match this trace with the peak inward current at 20 ms; and 3) the amplitude of each conventional I_{Na} was adjusted to the envelope of the peak inward currents at the falling phase. All records were collected from an identical cell. Lower graph shows the relationship between the duration (t) and the ratio of peak I_{Na} with and without conditioning pulse. Curves were drawn by eye.

by checking the h_∞-curve frequently during the experiment. As shown in Fig. 2, there is no shift of h_∞-curves up to 16 min (and up to 22 min in other experiments) after the start of the experiment.

Development of inactivation

The double-pulse method was employed. The first pulse was a conditioning pulse at a membrane potential of -35 or -20 mV for various durations. The second pulse was a 18 ms standard test pulse to 0 mV. One ms gap was set between the two pulses to achieve deactivation of the activation gate activated by the conditioning pulse. Care was exercised to achieve quick deactivation of the Na^+ channel by setting a holding potential of -100 mV. Data points were obtained from the peak of the current during the test pulses. We observed a marked delay of the onset of inactivation development at various membrane potentials of conditioning pulses as shown in Fig. 3. However, the time course of development



of inactivation measured by the double-pulse method was almost the same as that of the falling phase of I_{Na} by a single pulse. This delay was also observed in the more favorable experimental condition where $[Na^+]_o$ is reduced to 25% of the control (the external solution E_2) in order to decrease the effect of series resistance.

Recovery from inactivation

Time course of recovery from inactivation was obtained by a pair of depolarizing pulses to −20 mV. The procedures are diagrammed in Fig. 4. The first pulse had a duration of 40 ms, which is long enough to achieve complete inactivation of Na^+ channel. The second pulse was applied to estimate the amount of Na^+ conductance available after recovery for priming intervals of various durations at a membrane potential of −80, −90, or −100 mV. Figure 4 shows that the relationship between the peak of I_{Na} during the second pulse and the priming intervals is well fitted to a function of $\{1-\exp(-t/\tau)\}$.

In applying H-H formalism, data necessary for the processes of development of and recovery from inactivation at the same membrane potential were usually obtained separately with different protocols. Only when a preparation can induce

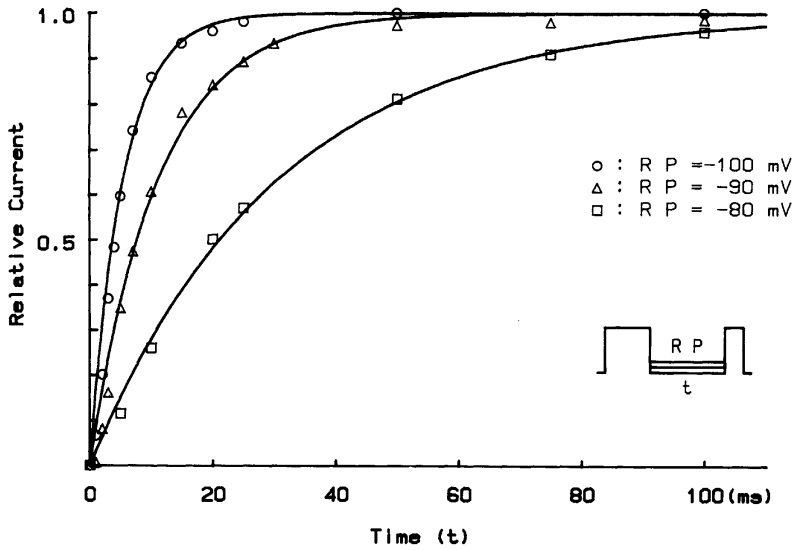


Fig. 4. Recovery from inactivation. Experimental protocol is shown in the inset at the lower right-hand corner. RP means the membrane potential in the period of recovery between the conditioning and test pulses. Ordinate indicates the amplitude of peak I_{Na} during the test pulse relative to that of I_{Na} at $t=650$ ms. Abscissa indicates the duration (t) of holding the membrane at RP. Data at three different RP's of −80, −90, and −100 mV are shown by squares, triangles, and circles, respectively. Curves were drawn for the equation $1-\exp(-t/\tau_r)$. The time constants (τ_r) are 48.54 ms for −80 mV, 14.65 ms for RP=90 mV, and 5.92 ms for RP=100 mV.

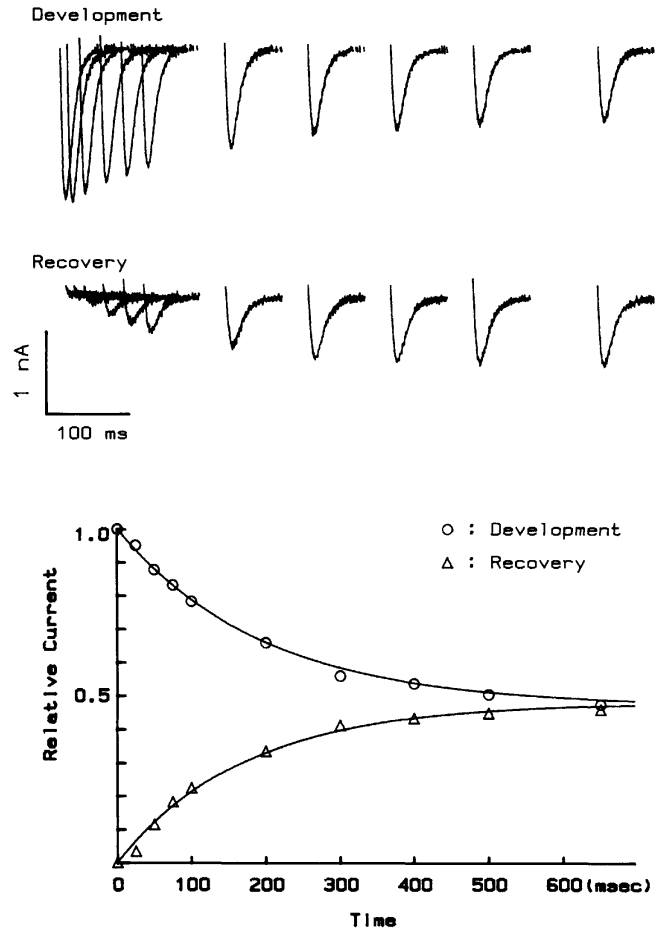


Fig. 5. Development of and recovery from inactivation at the same potential. Upper two panels are original records of I_{Na} for development of inactivation (top panel) and for recovery from inactivation (middle panel). Protocols for development and recovery are the same as those in Figs. 3 and 4, respectively. The membrane potential of the conditioning pulse (CP) and that for recovery (RP) were -65 mV. In the lower graph, ordinate indicates the ratio of peak I_{Na} with and without conditioning pulse. Abscissa indicates the duration (t) of both the conditioning pulse and the period for recovery. The upper curve was drawn according to the equation $(1-A)\times\exp(-t/\tau_h)+A$ where $A=0.47$ and $\tau_h=195$ ms and the lower curve to the equation $B\times\{1-\exp(-t/\tau_r)\}$, where $B=0.48$ and $\tau_r=172$ ms.

measurable I_{Na} around the threshold potential and the membrane potential can be controlled homogeneously with the voltage clamp method, the time constants for two processes can be obtained at an identical membrane potential. Under these conditions, the time constants for development (τ_h) and recovery (τ_r) at the

membrane potential of -65 mV were experimentally determined. In one case, τ_h and τ_r were calculated to be 195 and 172 ms, and in another case 215 and 190 ms, respectively. Two curves merged to a symptotic value of 0.48 which is close to the value estimated from the h_∞-curve at the concerned membrane potential. It is reasonable to assume that the relationship between the membrane potential and the time constant of inactivation can be expressed in a single function in this preparation, as described by SEYAMA and YAMAOKA (1988). Since the time courses of development of and recovery from the inactivated state are similar and consequently have similar time constants, the supposition (HAAS *et al.*, 1971) that there are two inactivation states having different time constants should be disqualified.

DISCUSSION

The main findings in this experiment are as follows: 1) a single exponential function is applicable to both the falling phase of I_{Na} and the time course of recovery from inactivation; 2) the relationship between the membrane potential and the time constant for inactivation can be expressed in a single function; 3) the *I-V* curve, instantaneous *I-V* relationship, and h_∞-curve, including these characteristics of the Na^+ channel, are in accord with the results obtained by SEYAMA and YAMAOKA (1988); and 4) a noticeable difference is recognized in that there is a clear delay of onset of inactivation. This property requires fundamental modifications of the previous kinetic scheme for the Na^+ channel in frog ventricular cells.

In taking account of all these observations, we propose a kinetic scheme in which there are several closed states prior to an open state and an inactivated state, and the inactivation process occurs independently both via the open state and via the last stage of closed states. The model we have selected is essentially the same as that of HORN and VANDENBERG (1984) as described below:

$$C_1 \overset{P_1}{\underset{}{\rightleftharpoons}} C_2 \overset{P_2}{\underset{}{\rightleftharpoons}} C_3 \overset{P_3}{\underset{}{\rightleftharpoons}} C_4 \overset{P_4}{\underset{}{\rightleftharpoons}} C_5 \overset{P_5}{\underset{}{\rightleftharpoons}} O \quad , \qquad P_6 \diagdown \quad \diagup P_7 \;\; I$$

$C_1, C_2, \cdots, C_5$: closed states; O: open state; I: inactivated state; $P_1, P_2, \cdots, P_7$: forward transitional rate constants.

The presence of a delay of onset of inactivation obliges us to propose several closed states before the open state and the inactivated state. Experimental evidence to support the notion of the presence of several closed states has been accumulated in *Myxicola* axon (GOLDMAN, 1976), squid axon (BEZANILLA and ARMSTRONG, 1977), rat pituitary gland (HORN and VANDENBERG, 1984), neonatal rat cardiac cells (KUNZE *et al.*, 1985), and canine cardiac Purkinje cells (FOZZARD *et al.*, 1987). Another experimental finding to limit the selection of the model for the Na^+ channel is that the time constants for development of and recovery from inactivation at the

same membrane potential are similar to each other. This finding requires one state of inactivation in this model. The time constant for the falling phase of I_{Na}, which indicates a change in state only from the open state to the inactivated state (see Fig. 1), and that for the development of inactivation, which indicates a change in state either from closed states or from the open state to the inactivated state (see Fig. 3), are coincident. This finding suggests that rate constants from both the open state and closed states to the inactivated state should be identical. The proposed model contains the assumption that when the channel is in a closed state it can move directly to the inactivated state without passing through the open state. ALDRICH *et al.* (1983) have shown that single channel records with no opening are related with the presence of the direct route from the closed states to the inactivated state.

Simulation of I_{Na} was performed in this model, in which the number of closed states and the transitional rate constants between each state were determined in such a way as to incorporate the experimental findings into the model and match the simulated curves with the data obtained experimentally. This simulation was

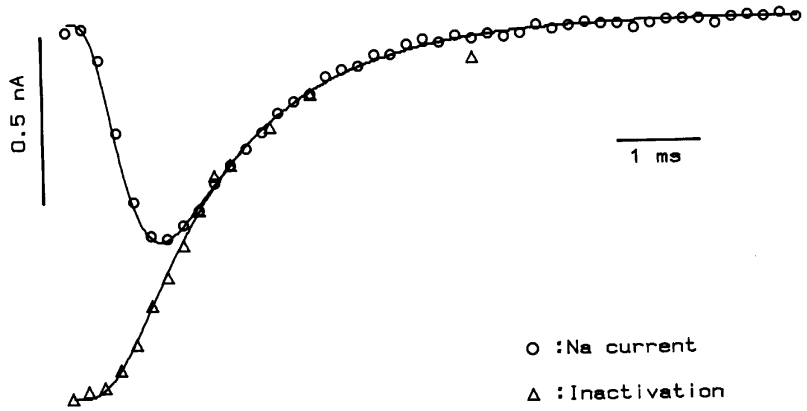


Fig. 6. Simulation of I_{Na} and development of inactivation. This simulation was performed in the proposed model for both I_{Na} and development of inactivation at the membrane potential of -20 mV. The simulated curves were drawn as solid lines. At initiation, C_1 was set to one and other states were zero. Probability of each state was calculated by solving the differential equations using the Runge-Kutta method. The curve for I_{Na} was obtained as the change of probability of O. That for the development of inactivation was obtained by the equation of (1 − probability of I). The forward rate constants between different states were chosen to be $P_1 = P_2 = P_3 = P_4 = 6.55\ ms^{-1}$, $P_5 = 8.20\ ms^{-1}$, and $P_6 = P_7 = 0.71\ ms^{-1}$. The original record of I_{Na} in response to the depolarizing pulse to -20 mV from a holding potential of -100 mV is shown by open circles. That of the development of inactivation, shown by open triangles, was obtained with the double-pulse method when CP = -20 mV in Fig. 3. The falling phase of I_{Na} is well expressed by a single exponential function, having a time constant of $\tau_h = 1.47$ ms.

done for both I_{Na} and development of inactivation at -20 mV where the number of closed states is five and the rate constant between each closed state is identical. The rate constants for inactivation (C_5 to I and O to I) were set to be the same. All the backward rate constants were set to 0, because no practical recovery from inactivation could be observed at this membrane potential. Simulation successfully reproduced the delay of onset of inactivation as well as the time course of the falling phase of I_{Na} in a single exponential function.

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REFERENCES

ADELMAN, W. J., Jr. and SENFT, J. P. (1968) Dynamic asymmetries in the squid axon membrane. *J. Gen. Physiol.*, **51**: 102s–114s.

ALDRICH, R. W., COREY, D. P., and STEVENS, C. F. (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature*, **306**: 436–441.

BENNDORF, K., BOLDT, W., and NILIUS, B. (1985) Sodium current in single myocardial mouse cells. *Pflügers Arch.*, **404**: 190–196.

BEZANILLA, F. and ARMSTRONG, C. M. (1977) Inactivation of the sodium channel I. Sodium current experiments. *J. Gen. Physiol.*, **70**: 549–566.

BODEWEI, R., HERING, S., LEMKE, B., ROSENSHTRAUKH, L. V., UNDROVINAS, A. I., and WOLLENBERGER, A. (1982) Characterization of the fast sodium current in isolated rat myocardial cells: Simulation of the clamped membrane potential. *J. Physiol. (Lond.)*, **325**: 301–315.

BROWN, A. M., LEE, K. S., and POWELL, T. (1981a) Voltage clamp and internal perfusion of single rat heart muscle cells. *J. Physiol. (Lond.)*, **318**: 455–477.

BROWN, A. M., LEE, K. S., and POWELL, T. (1981b) Sodium current in single rat heart muscle cells. *J. Physiol. (Lond.)*, **318**: 479–500.

BUSTAMANTE, J. O. and MCDONALD, T. F. (1983) Sodium currents in segments of human heart cells. *Science*, **220**: 320–321.

CACHELIN, A. B., DE PEYER, J. E., KOKUBUN, S., and REUTER, H. (1983) Sodium channels in cultured cardiac cells. *J. Physiol. (Lond.)*, **340**: 389–402.

FOZZARD, H. A., HANCK, D. A., MAKIELSKI, J. C., SCANLEY, B. E., and SHEETS, M. F. (1987) Sodium channels in cardiac Purkinje cells. *Experientia*, **43**: 1162–1168.

FRANKENHAEUSER, B. and HODGKIN, A. L. (1957) The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.)* **137**: 218–244.

GOLDMAN, L. (1976) Kinetics of channel gating in excitable membranes. *Q. Rev. Biophys.*, **9**: 491–526.

HAAS, H. G., KERN, R., EINWÄCHTER, H. M., and TARR, M. (1971) Kinetics of Na inactivation in frog atria. *Pflügers Arch.*, **323**: 141–157.

HESS, P., LANSMAN, J. B., and TSIEN, R. W. (1986) Calcium channel selectivity for divalent and monovalent cations. *J. Gen. Physiol.*, **88**: 293–319.

HODGKIN, A. L. and HUXLEY, A. F. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)*, **117**: 500–544.

HORN, R. and VANDENBERG, C. A. (1984) Statistical properties of single sodium channels. *J. Gen. Physiol.*, **84**: 505–534.

KUNZE, D. L., LACERDA, A. E., WILSON, D. L., and BROWN, A. M. (1985) Cardiac Na currents and the inactivating, reopening, and waiting properties of single cardiac Na channels. *J. Gen. Physiol.*, **86**: 691–719.

MAKIELSKI, J. C., SHEETS, M. F., HANCK, D. A., JANUARY, C. T., and FOZZARD, H. A. (1987) Sodium current in voltage clamped internally perfused canine cardiac Purkinje cells. *Biophys. J.*, **52**: 1–11.

MARQUARDT, D. W. (1963) An algorithm for least-square estimation of non-linear parameters. *J. Soc. Ind. Appl. Math.*, **11**: 431–441.

MATSUKI, N. and HERMSMEYER, K. (1983) Tetrodotoxin-sensitive Na^+ channels in isolated single cultured rat myocardial cells. *Am. J. Physiol.*, **245**: C381–C387.

MITSUIYE, T. and NOMA, A. (1987) A new oil-gap method for internal perfusion and voltage clamp of single cardiac cells. *Pflügers Arch.*, **410**: 7–14.

PATLAK, J. B. and ORTIZ, M. (1985) Slow currents through single sodium channels of the adult rat heart. *J. Gen. Physiol.*, **86**: 89–104.

SEYAMA, I. and YAMAOKA, K. (1988) A study of the electrical characteristics of sodium currents in single ventricular cells of the frog. *J. Physiol. (Lond.)*, **401**: 257–275.