THE LINK BETWEEN SODIUM ACTIVATION AND INACTIVATION

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A modification of a mathematical model of the node of Ranvier based on the postulated dependence of the rate of inactivation (h) on the rate of activation (m) is suggested. The model reproduces sodium fluxes under voltage and action potential clamp conditions and also the experimentally obtained delay in the development of inactivation. The model interprets the experimentally observed initial decline in the peak values of I_{Na} (I_{Na}^P) after withdrawal of a short depolarizing stimulus as a true continuation of inactivation (a decline in h) unlike the Hodgkin–Huxley model, in which this decline is explained by the powerful dependence of I_{Na}^P on the original m. The model predicts a decrease in h in response to a factor increasing m without changing the membrane potential. An increase in the level of inactivation at the resting potential was found in experiments on a node of Ranvier on the prolonged "tail" of increased sodium conductance arising after withdrawal of a depolarizing stimulus in an external medium containing veratrine.

The writers have shown previously that inactivation of the sodium channels in the membrane of the Ranvier node develops after an initial delay, the duration of which correlates with the steepness of rise of sodium conductance. The existence of this delay, which has also been found with other objects [3], casts doubt upon the view expressed in the Hodgkin–Huxley [4] equations concerning the isolated character of inactivation.

In this paper a model of the changes in sodium conductance which takes this feature into account is examined, and experiments carried out in connection with certain predictions of the model are discussed.

EXPERIMENTAL METHOD

Isolated nerve fibers from the frog Rana ridibunda were used in experiments by the voltage clamp technique described previously [1].

EXPERIMENTAL RESULTS AND DISCUSSION

The design of the model was based on the views of Hodgkin and Huxley regarding the existence of the two processes: activation and inactivation:

$$I_{Na} = \bar{g}_{Na} m^3 h (E - E_{Na}), \tag{1}$$

where I_{Na} is the sodium current; $\bar{g}_{Na}$ the maximal sodium conductance; m and h are variables describing the processes of sodium activation and inactivation, respectively; E is the membrane potential; and E_{Na} the equilibrium sodium potential.

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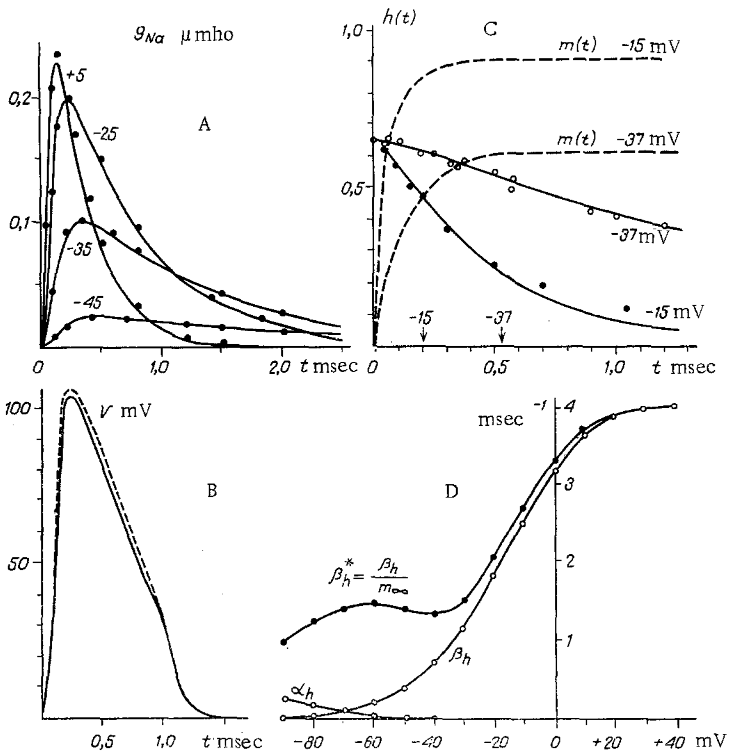

Fig. 1. Basic characteristics of the model and its predictions: A) changes in $\bar{g}_{Na}$ in time for various clamped voltages, calculated by the Hodgkin–Huxley equations in Hille's modification [8] for a node (continuous lines) and predictions of the new model for the same conditions (shown by dots); numbers denote values of membrane potential in millivolts; B) action potential calculated by Hodgkin–Huxley equations (broken line) and by Eqs. (1)-(3); C) development of inactivation in time with a shift of membrane potential from the resting potential (-75 mv) toward depolarization. Continuous lines represent the course of changes in h for two values of the potential (-37 and -15 mv), calculated by Eqs. (1)-(3) for 20°C. Broken lines show changes in m for corresponding values of potentials. Experimental points were obtained by using the maximal value of the I_{Na} derivative as a measure of h. Fiber 18.5.72. Intertrial interval 250 µsec. Temperature 20°C. Experimental points given with a correction for the increase in h in the intertrial interval with a time constant of 6 msec; D) graph of velocity constants of inactivation as a function of membrane potential.

Since this paper is devoted to the study of inactivation, the description of activation suggested by Hodgkin and Huxley [4] was left unaltered:

$$m = \alpha_m \cdot (1 - m) - \beta_m \cdot m, \qquad (2)$$

where α_m and β_m are velocity constants of the process m.

The new description of the inactivation process must satisfy two requirements: first, it must give a delay in the development of inactivation and, second, it must describe (just as well as the Hodgkin–Huxley model) the kinetics of the decrease in h at large values of time and the shape of the descending phase of g_{Na} (t) respectively. The equation is

$$\dot{h} = \alpha_h(1 - h) - \beta_h^* \cdot m \cdot h, \qquad (3)$$

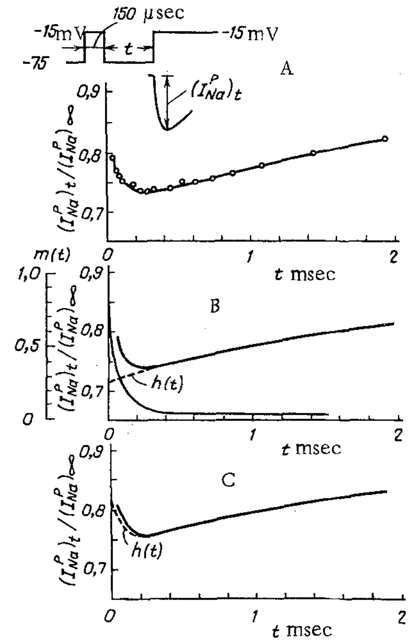

Fig. 2

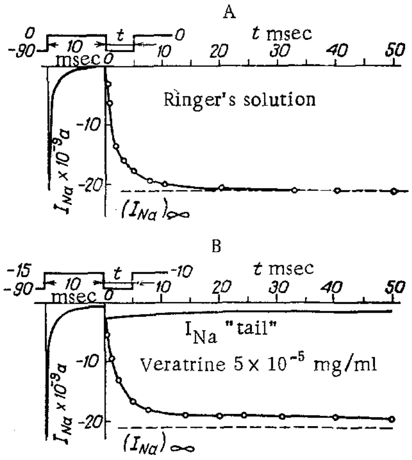

Fig. 3

Fig. 2. Changes in peak values of $I_{Na}(I^P_{Na})$ developing in response to test stimuli applied with a varied delay t after the end of the short depolarizing stimulus: A) curve showing I^P_{Na} as a function of t obtained experimentally. Scheme of experiment illustrated above. Duration of conditioning stimulus 150 µsec. Numbers denote values of membrane potential. Fiber 8.1.73. Temperature 20°C; B) curve of I^P_{Na} versus t calculated from Hodgkin–Huxley equations (thick line) for the same conditions as in A. Course of changes in h shown by broken line, course of changes in m in intertrial interval shown by thin line; C) the same as in B but calculated by Eqs.(1)-(3).

Fig. 3. Experimental curves of withdrawal from inactivation after 10 msec of depolarizing stimulus obtained from peak values I^P_{Na} in response to test stimuli. In each part of this figure scheme of experiment shown above graph (numbers denote values of membrane potential in millivolts), on left of ordinate values of I_{Na} developing during the action of conditioning stimulus. Broken line gives value of peak I_{Na} in absence of conditioning stimulus. Fiber 14.11.72. Temperature 22°C. A) Node in Ringer's solution; B) external solution contains veratrine (5×10^{-5} mg/ml). Continuous line without points shows "tail" of sodium current after repolarization.

where α_h has the same significance as in the Hodgkin–Huxley model and $\beta^*_h = \beta_h / m_\infty$ satisfies both requirements.[†] It gives a delay in the development of inactivation (Fig. 1C), and at times when m reaches its stationary value m the equation changes into the corresponding Hodgkin–Huxley equation. Calculations carried out with numerical results for a standard node [7] showed that the model reproduces the sodium currents under voltage and action potential clamp conditions very well (Fig. 1: A and B).

The new velocity constant β^*_h is less highly dependent on potential than β_h (Fig. 1D) and is still quite high at the resting potential or even at high negative values of E. Accordingly the model predicts the prolongation of inactivation (under certain conditions) even after the cessation of depolarization. In fact, if at the time of repolarization h is not too small while m is sufficiently large, it is found that $\beta^*_h \cdot m \cdot h > \alpha\,(1-h)$ and, consequently, there will be a further decline in h (Fig. 2C). This observation enables a fundamentally

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[†] Here and subsequently Hodgkin–Huxley equations for the node of Ranvier are implied [5, 6]. For the calculations these equations were used in Hille's modification [7].

different interpretation from that given by the Hodgkin–Huxley equations to be suggested for the character of the changes in the peaks of the sodium current (I^P_{Na}) observed experimentally in response to test stimuli applied at various time intervals t after withdrawal of a short depolarizing stimulus. The experimentally obtained curve of I^P_{Na} as a function of t is shown in Fig. 2A. Clearly the increase in I^P_{Na} connected with the withdrawal of the sodium channels from inactivation (increase in h) is clearly preceded by a short time interval in which I^P_{Na} falls. Both models reproduce this phenomenon well (Fig. 2: B and C), but for completely different reasons: in the Hodgkin–Huxley model the initial decrease in I^P_{Na} is the result of a decrease in m after withdrawal of the conditioning stimulus, while in the modified model it is the result of true prolongation of inactivation (a decrease in h). This is explained as follows. In the Hodgkin–Huxley model the peak value g^P_{Na} and, consequently, I^P_{Na} is strongly dependent not only on the value of h at the time of application of the test stimulus (h_t), but also on the initial value of m (m_t):

$$I^P_{Na}/(I^P_{Na}) = h_t(1 - m_t/m_\infty)^{-\tau_m/\tau^h} . \tag{4}$$

For this reason the rapid decrease in m after the end of the conditioning stimulus leads to a temporary drop in I^P_{Na} despite the continuous increase in h (Fig. 2B). In the new model, however, I^P_{Na} depends to a much lesser degree on m_t:

$$I^P_{Na}/(I^P_{Na})_{max} = h_t[(1 - m_t/m_\infty)\exp(m_t/m_\infty)]^{-\tau_m/\tau_h} \tag{5}$$

and the decrease in I^P_{Na} reflects the changes in h quite well (Fig. 2C).

The fact that I^P_{Na} is only slightly dependent on m_t (when $m_t < 2/3\, m_\infty$ for a particular test potential) in this model is explained by the connection between h and m, which leads to initial delay in the decline of h at small values of m. Evaluation of the relative decrease in I^P_{Na} by Eq. (5) shows that only 2.5% of the 8% obtained experimentally (Fig. 2A) can be attributed to differences in the initial values of m_t. The existence of delay in the development of inactivation, where it is the result of a connection between h and m, thus necessarily implies only a slight dependence of I^P_{Na} on the initial value of m_t. In that case the experimentally observed initial decrease in I^P_{Na} after the end of depolarization must be attributed chiefly to a true prolongation of inactivation.† The prolongation of inactivation, however, can take place only if the velocity constant of transition into the inactivated state at the resting potential is significantly greater than the velocity constants of the reverse change. In the new model this condition, as has already been stated above, is satisfied automatically as a result of the introduction of a normalizing factor $1/m_\infty$ into Eq. (3) to give adequate description of the descending limb of $\bar{g}_{Na}(t)$ under voltage clamp conditions.

In connection with the above remarks it was interesting to create artificial conditions such that the sodium conductants would be increased without a shift of membrane potential. These conditions could be obtained in the presence of veratrine in the external medium, for during its action [7, 9] a prolonged (time constant 200-600 msec) "tail" of increased $\bar{g}_{Na}$ is observed after withdrawal of the depolarizing stimulus. Experimental curves of withdrawal from inactivation after 10 msec of the depolarizing stimulus are shown in Fig. 3. In the presence of veratrine inactivation was quickly removed (with the same time constant as in the control Ringer's solution), but the quasisteady-state level of inactivation reached was found to be increased; this increased level of inactivation persisted as long as the $\bar{g}_{Na}$ "tail". To explain this result within the framework of the new model it is unnecessary to make any additional assumptions regarding the action of veratrine on the sodium pore beyond that it maintains the pores with which it has succeeded in binding during the action of the depolarizing stimulus in the activated state even after repolarization. Since β^*_h at the initial potential was high enough, a certain proportion of activated pores will return into the inactivated state. In the case of a model with independent values of h and m, to explain this fact it would be necessary to assume that veratrine causes much more profound changes in the sodium pore.

The fact that besides the action potential, ionic currents, and delay in the development of inactivation, the model reproduces the decline in h after withdrawal of the short depolarizing stimulus as well as the increased level of inactivation on the "tail" of the increased $\bar{g}_{Na}$, justifies the hope that as a first approximation it will truly reflect the character of the connection between activation and inactivation. Assuming that the constant α_h (just like β^*_h) does not become zero at any values of the membrane potential, inactivation will never be complete. The absence of a peak of the inward current in response to the test stimulus is evidence simply that inactivation has reached saturation, and the solution of the problem of whether the resul-

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† The prolongation of inactivation after withdrawal of the depolarizing stimulus has also been found in the potassium system of mollusk giant neurons [2, 8]. The results of the present investigation suggest that inactivation is dependent on activation in the potassium system also.

tant ionic current in this case contains or does not contain a component of the inward sodium current depends on the writers' assumption regarding the leakage current and the potassium current. No evidence conflicting with the hypothesis of incomplete activation could be found; indeed, the facts indicate that this correctly reflects the true state of affairs [10].

It must be emphasized that the suggested model is still purely formal, and that very many different physical models reflecting the basic assumption regarding the character of the connection between activation and inactivation could be devised. It is to be hoped that the attempt to explain all the known effects of veratrine and the future experiments with it will lead to fresh progress in physical modeling.

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