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ROLE OF ION EXCHANGE PROCESSES IN THE MECHANISM OF SODIUM CONDUCTANCE CHANGES IN EXCITABLE MEMBRANES

S. N. FISHMAN, V. A. CHERNEIKIN and M. V. VOLKENSTEIN

Institute of Molecular Biology, U.S.S.R. Academy of Sciences, Moscow (U.S.S.R.)

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SUMMARY

A theoretical investigation of the influence of K^+ in the surrounding solution on the Na^+ inactivation of excitable membranes was carried out. A scheme was proposed which involves ion exchange processes at the membrane site connected with conductance. The theory explains the decrease of the inactivation parameter, h_∞, with increase of K^+ concentration in solution, C_K, as well as the increase of inactivation time constant, τ_h, with increase of C_K. It is suggested that Na^+ takes part in the activation of Na^+ conductance, and Na^+ permeability is calculated as a function of Na^+ concentration in solution.

In previous papers[1,2] a physicochemical model of Na^+ conductance changes in the processes of activation and inactivation has been suggested. The model is based on the analysis of experimental data concerning the influence of electric field and concentration of Ca^{2+} in the external media on the conductance of nerve fibers. It has been suggested that membrane sites can exist in conductable (Y) and non-conductable (X) states. In the X state the site can bind Ca^{2+}, forming the state $S \equiv XCa$, and from the Y state the site can turn into the inactivated state Z (Fig. 1A).

The transitions $X \rightleftarrows Y$ and $S \rightleftarrows Z$ are connected with membrane structure transformation. In this transformation electric field produces work $n\delta EF$ per mole of sites (F, Faraday number, n, charge, driven by the field, E, membrane potential counted from potential of the outer membrane surface, δ that part of the membrane potential which acts on the charge). Equilibrium constants K_2 and K_4 ($K_i = k_i'/k_i$) depend on the field E:

$$\begin{aligned} K_2 &= q_2 \exp\{-n\delta EF/RT\} \\ K_4 &= q_4 \exp\{n\delta EF/RT\} \end{aligned} \tag{1}$$

The main experimental effects, studied quantitatively, depend only on one theoretical parameter, $n\delta$. Determining its value from one type of experiment, one can foretell the results of other experiments quantitatively. Evaluation of the parameter $n\delta$ gives its value as about 4.

For a better understanding of the physical processes taking place in the mem-

brane under the action of electric field, one has to study many physical characteristics of the membrane, which are changing simultaneously with the changes of membrane conductance. Cohen *et al.*[3,4] studied changes in light scattering, Tasaki *et al.*[5] studied changes in fluorescence, and Berestovski *et al.*[6] studied changes in birefringence of nerve fiber membranes caused by the appearance of membrane action potential. However, these studies are now at the stage of collection of experimental data.

At the same time there exists the possibility of studying the processes determining the changes of Na^+ conductance in a different way, based on a large number of electrophysiological observations with detailed analysis of the action of different ions on the membrane conductance. On the grounds of such observations, Tasaki and his co-workers (see ref. 7) put forward the idea about the leading role of ion exchange processes in the mechanism of action potential generation. At present, apparently, one can make a quantitative theoretical analysis of the physicochemical model that involves ion exchange reactions. Voltage clamp experiments on the changes of Na^+ conductance ought to be the basis of such analysis, which is the purpose of this paper. First steps in that direction were made in previous work[1], where the role of the pH of the external solution was analyzed. The conclusions obtained are in agreement with experimental data[8,9].

Adelman and Palti[10] have studied the influence of K^+ concentration, C_K, in the external media on the inactivation of Na^+ conductance. Dependence of the peak Na^+ current, I_{Na}, on the magnitude of the prepotential, E_0, can be normalized in two ways in this case:

$$h_\infty \equiv I_{Na}(C_K,E_0)/\lim_{E_0\to-\infty} I_{Na}(0,E_0) \tag{2}$$

$$h'_\infty \equiv I_{Na}(C_K,E_0)/\lim_{E_0\to-\infty} I_{Na}(C_K,E_0) \tag{3}$$

The sign ∞ means that the membrane has been maintained under the prepotential conditions for a sufficiently long time. Physically, h means "the fraction of the Na^+-carrying system which is rapidly available for carrying Na^+ when the membrane is depolarized" (see ref. 11). In a previous paper[10] the dependence of h_∞, h'_∞ and the kinetic constant τ_h on E_0, when using different C_K, has been obtained.

It appears from these observations that h_∞ decreases with the rise in C_K. This means that the increase in concentration of K^+ in the bathing solution leads to a decrease in the number of sites in states S, X and Y. The effect remains clear when the membrane is strongly hyperpolarized, that is when the part of the sites in state Z is practically negligible. It can be suggested that some physicochemical state exists in the membrane which is determined by K^+. Transition from this state into S or X is not connected with the work of charge in the electric field and is slow. This state is referred to as L, and these processes are represented in Scheme B (Fig. 1).

When the membrane is strongly hyperpolarized ($E_0\to-\infty$), all sites are distributed between states X, S and L. This distribution can be obtained from the system of equations.

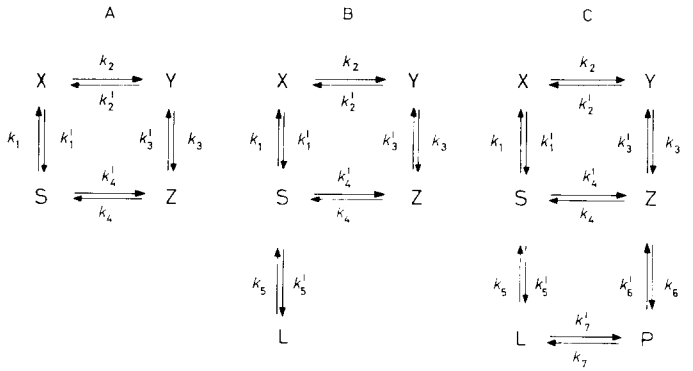

Fig. 1. Modification of the scheme describing Na^+ conductance inactivation. A, scheme describing the action of Ca^{2+} and electric field. B, modification that is needed for describing the decrease of

$$\lim_{E_0 \to -\infty} h'_\infty$$

when K^+ concentration increases in the solution. C, modification that is needed for describing all the effects of K^+ concentration in solution.

$$x_{-\infty} + s_{-\infty} + l_{-\infty} = 1$$
$$k_1 s_{-\infty} = k'_1 x_{-\infty}$$
$$k_5 l_{-\infty} = k'_5 s_{-\infty} \tag{4}$$

From the definition of the physical sense of h_∞ it is clear that

$$\lim_{E_0 \to -\infty} h_\infty = x_{-\infty} + s_{-\infty} = 1 \Big/ \left(1 + \frac{K_1}{1 + K_1} K_5\right) \tag{5}$$

where $K_i = k_i'/k_i$. In the analysis of the process of Na^+ conductance activation it has been established that the reaction $X \rightleftarrows S$ ought to be displaced to the right, that means $K_1 \gg 1$. Using this condition we obtain from Eqn 5:

$$\lim_{E_0 \to -\infty} h_\infty = 1/(1 + K_5) \tag{6}$$

The site binds Ca^{2+} in the state S. The experiment of Adelman and Palti[10] shows that the part of the sites in state L increases with the increase of C_K. Thus, it follows that one can treat the transition S→L as an exchange of Ca^{2+} to K^+ (that is $XCa \rightarrow XK_2$). In this case the constant K_5 must be proportional to C_K^2 ($K_5 \equiv \eta C_K^2$) and

$$\lim_{E_0 \to -\infty} h_\infty = 1/(1 + \eta C_K^2) \tag{7}$$

Fig. 2 shows Eqn 7 graphically. The value of the parameter η was $9 \cdot 10^{-4}$. Points correspond to the experimental data taken from a previous paper[10]. The dependence is non-linear and the agreement between the theoretical curve and experimental data, obtained with the use of one fitting parameter, supports the theoretical assumptions.

Analysis analogous to that described above has shown that Scheme B (Fig. 1) cannot explain the experimental dependence h_∞' (C_K). The experiment gives, in the range of concentrations $0 < C_K < 100$ mM, the following:

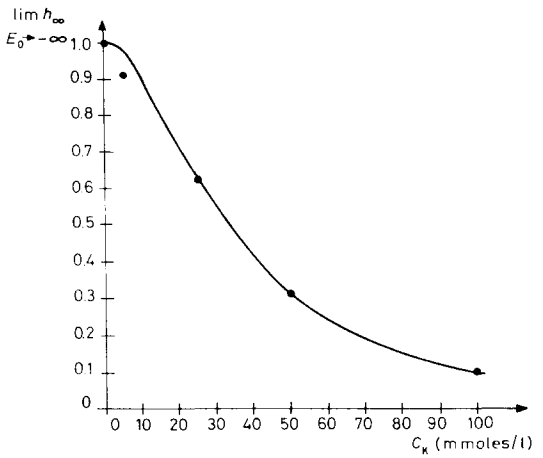

Fig. 2. Dependence of limit magnitude of parameter h_∞ under large membrane hyperpolarization on the K^+ concentration. The curve is drawn according to the theoretical Eqn 7 with the value of the fitting parameter $\eta = 9 \cdot 10^{-4}$. The points were taken from the experimental data of Adelman and Palti[10].

$$dh'_\infty/dC_K < 0 \tag{8}$$

The opposite sign of the derivative follows from Scheme B for any given C_K. Eqn 8 can be obtained theoretically only through introduction into the scheme of some other inactivated K^+ state in which the site can exist when the membrane is depolarized. However, one cannot assume that this is the Z state because, in that case, the dependence of h_∞ on prepotential disappears when $C_K \to O$. Let us refer to this state as P. The scheme then must be of the type C (Fig. 1). We have shown above that in state L the site binds two K^+. We cannot determine from the available experimental data the number of K^+ the site binds in the P state. Let us consider the case where this number is equal to 2, although the results of analysis of these experimental data do not change appreciably if the number is equal to 1. For discrimination of these variants some supplementary experiments are needed.

The states Z, L and P together comprise the group E of inactivated states, the transition of the sites from this group of states to the one of the activated states X, Y, S (group H) is slow.

Let us continue the analysis further using the method described in a previous paper[2]. The maximal value to which the part of the sites in state Y tends when the test is carried out will be denoted as y^*. As the equilibrium is rapidly established between the states of H group, y^* must be close to the corresponding equilibrium magnitude under the condition where that part of the sites in the F states remains unchanged:

$$y^* \cong \frac{1 - (z_0 + l_0 + p_0)}{1 + K_2 + K_1 K_2} \tag{9}$$

where z_0, l_0 and p_0 are the parts of the sites in states Z, L and P, respectively, corresponding to the prepotential state. From the equilibrium conditions:

$$x_0 = K_{20}y_0; \quad y_0 = K_3 z_0; \quad z_0 = K_{40}s_0$$
$$s_0 = K_1 x_0; \quad l_0 = K_5 s_0; \quad p_0 = K_{70} l_0 \tag{10}$$

and conservation condition:

$$x_0 + y_0 + z_0 + s_0 + p_0 + l_0 = 1 \tag{11}$$

one can find y^* in the following form, where subscript "0" denotes the parameters dependent on the prepotential, E_0

$$y^* = \frac{1}{1 + K_2 + K_1 K_2} \cdot \frac{1}{1 + \chi K_{40} + \chi\zeta C_K{}^2 + \chi\pi C_K{}^2 K_{40}} \tag{12}$$

where

$$K_5 \equiv \zeta C_K{}^2; \quad K_6{}^{-1} \equiv \pi C_K{}^2; \quad K_1/(1 + K_1) \equiv \chi \tag{13}$$

Eqn 12 was obtained by taking into account that the equilibrium state of reaction $Y \rightleftarrows Z$ is shifted to Z, *i.e.* $K_3 \ll 1$, which is the necessary condition for the appearance of the peak of Na^+ conductance. With the help of Eqn 1 we obtain from Eqn 12

$$\lim_{E_0 \to -\infty} y^* = \frac{1}{1 + K_2 + K_1 K_2} \cdot \frac{1}{1 + \chi\zeta C_K{}^2}$$

$$\lim_{\substack{E_0 \to -\infty \\ C_K \to 0}} y^* = \frac{1}{1 + K_2 + K_1 K_2} \tag{14}$$

The value of γ^* is proportional to the peak Na^+ current, I_{Na}. Using Definitions 2 and 3 and Eqns 12 and 14 we obtain:

$$h_\infty = 1/(1 + \chi\zeta C_K{}^2 + \pi\chi C_K{}^2 K_{40} + \chi K_{40}) \tag{15}$$

$$h'_\infty = (1 + \chi\zeta C_K{}^2)/(1 + \zeta\chi C_K{}^2 + \pi\chi C_K{}^2 K_{40} + \chi K_{40}) \tag{16}$$

It can be shown that the function h'_∞ (C_K) described by Eqn 16, satisfies Condition 8, if:

$$K_6{}^{-1}(1 + K_1{}^{-1}) > K_5 \tag{17}$$

or as $K_1 \gg 1$

$$K_6{}^{-1} > K_5 \tag{18}$$

This restriction corresponds to the requirement that the absorption of K^+ at the depolarized membrane is energetically more profitable than at the membrane under resting potential.

Let us now consider kinetic effects. If the transitions between the states of the H and F group are much slower than those occurring within each group, the kinetics of inactivation are determined by the transition between these groups and can be described by a one-exponential curve. These conclusions agree with the experimental data. According to this adiabatic approximation one can write

$$h \equiv x + y + s = (1 + K_1^{-1} + K_1^{-1}K_2^{-1})s = (1 + K_2 + K_1K_2)y \tag{19}$$

$$f \equiv l + p + z = (1 + K_6^{-1} + K_4^{-1}K_5)z = (1 + K_7 + K_6K_7)l \tag{20}$$

$$h + f = 1 \tag{21}$$

$$\dot{h} = -k_3y - (k'_4 + k'_5)s + (k'_3 + k_4)z + k_5l \tag{22}$$

Using Eqns 19–21 we can rewrite Eqn 22 in the form:

$$\dot{h} = -\frac{k_3 + (k'_4 + k'_5)K_1K_2}{1 + K_2 + K_1K_2}h + \frac{k'_3 + k_4 + k_5K_4^{-1}K_5}{1 + K_6^{-1} + K_5K_4^{-1}}(1 - h) \tag{23}$$

Eqn 23 has a solution of the form

$$h = a + b\exp(-t/\tau_h) \tag{24}$$

where time constant τ_h is equal to

$$\tau_h = \left[\frac{k_3 + (k'_4 + k'_5)K_1K_2}{1 + K_2 + K_1K_2} + \frac{k'_3 + k_4 + k_5K_4^{-1}K_5}{1 + K_6^{-1} + K_5K_4^{-1}}\right]^{-1} \tag{25}$$

When the right-hand part of Eqn 23 is equal to zero, we obtain the expression for h_∞, the equilibrium value of h. This expression can be written in the same form as Eqn 15. Eqn 25 can be expressed in the following form:

$$\tau_h = h_\infty/\alpha_h \tag{26}$$

where

$$\alpha_h = \frac{k'_3 + k_4 + k'_5K_4}{1 + K_6^{-1} + K_5K_4^{-1}} \tag{27}$$

If the dependence of the constants on the membrane potential (Eqn 1) and concentration C_K (Eqn 13) is taken into account, it can be shown qualitatively that the function τ_h (E) is described by a curve with a maximum, the value of which increases with the increase of C_K, as has been observed in the experiments of Adelman and Palti[10].

We can conclude that the substitution of Ca^{2+} by $2K^+$ transforms the site to the L state, from which the transition to another "potassium" state, P, is possible. This transition is connected with the work of the charge fixed in the membrane in the electric field.

We have built a theoretical model with the intention of explaining the experimental data. The model allows us to forecast a number of phenomena that have not been observed before. The participation of external K^+ in the two inactivated states permits us to suppose that the X and Y states connected with the activation of Na^+ conductance are analogous "sodium" states and therefore the reaction $S \rightleftarrows X$ describes the exchange of Ca^{2+} with $2Na^+$. In this case the value of Na^+ concentration in the external solution must influence the membrane permeability, P_{Na}.

If the site binds two Na^+ in the state X and μ Na^+ in the state Y, the constants

K_1 and K_2 can be described in the following way:

$$K_1 = \bar{K}_1 C_{Na}^{-2}; \quad K_2 = \bar{K}_2 C_{Na}^{2-\mu} \tag{28}$$

Under the conditions of large prehyperpolarization ($z_0 \cong p_0 \cong 0$) and small C_K ($l_0 \cong 0$), we obtain from Eqn 9 with the help of Definition 28

$$y^* = (1 + \bar{K}_2 C_{Na}^{2-\mu} + \bar{K}_1 \bar{K}_2 C_{Na}^{-\mu})^{-1} \tag{29}$$

Na^+ permeability of the system should be proportional to y^* and, if our hypothesis is correct, the dependence of P_{Na} on C_{Na} is described by Formula 29. If, for example, $\mu = 2$, then

$$P_{Na} = k(1 + \bar{K}_2 + \bar{K}_1 \bar{K}_2 C_{Na}^{-2})^{-1} \tag{30}$$

where k is the proportionality coefficient.

A corresponding curve is shown in Fig. 3. If C_{Na} is large enough, we obtain the asymptotic part of the curve and P_{Na} does not practically depend on C_{Na}. We have to examine the relation between P_{Na} and C_{Na} under small Na^+ concentrations.

On the basis of this hypothesis concerning the role of Na^+ in the activation of Na^+ conductance we can make an assumption about molecular mechanism of the ion transfer. Let physical process X→Y change the state of the site in such a way that the binding of one or two Na^+ with the site in the state X becomes weak; the ion then moves in the direction of the electrochemical potential gradient, another ion from solution takes its place and so on. Thus, the site in the Y state is the center of ion transfer.

Using this scheme one can analyse the action of physicochemical and pharmacological agents (toxins, anesthetics *etc.*). Li^+, NH_4^+, guanidine and other ions have apparently an affinity to the site similar to that of Na^+; they replace Na^+ easily and are transported by Na^+ channels. Tetrotoxin contains a guanidine group in its structure and can also replace Na^+ on the site, but because of its specific electronic structure it cannot be transferred and strongly binds with the site, blocking its conductance.

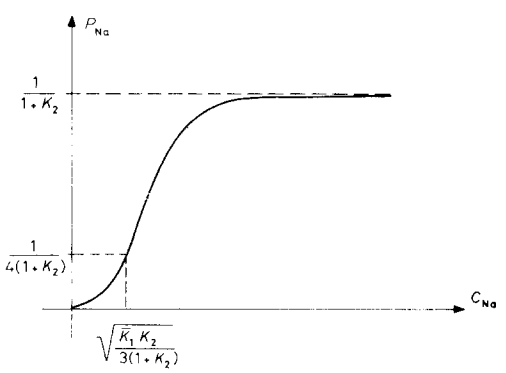

Fig. 3. Dependence of the Na^+ permeability on Na^+ concentration predicted for the case where the site binds two Na^+ in the X and Y states.

We have discussed only the Na^+ permeability of the membrane. The structure of Scheme C (Fig. 1) is such that one can assume that the site in the P state has the ability to conduct K^+. At present many authors (see *e.g.* refs 11 and 12) consider all available experimental data from the point of view of the existence of two independent conductance channels (Na^+ and K^+ channels). However, these data can be treated qualitatively with the help of our scheme, *i.e.* when in the P state the site is K^+ conductive. This problem needs a quantitative study which we are trying to perform at the present time.

Therefore a role for the ion exchange processes in the phenomena connected with Na^+ conductance changes in the excitable membranes can now be given. The sites determining membrane conductance can exist in two physical states. The work of charge in the electric field is performed during the transition from one state to another. One of these states is stabilized by Ca^{2+} (and perhaps by other bivalent cations). By the substitution of Ca^{2+} by Na^+ or K^+ the site obtains the ability to go rapidly into another state; this results in high Na^+ conductance if Na^+ is bound to the site, and binding of K^+ transforms the site into an inactivated state (as regards Na^+ conductance).

Apparently, this scheme can explain the action of other ions on the Na^+ conductance, the action of tetrodotoxin and saxytoxin for example.

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REFERENCES

1 Fishman, S. N., Khodorov, B. I. and Volkenstein, M. V. (1971) *Biochim. Biophys. Acta* 225 1
2 Fishman S. N. and Volkenstein, M. V. (1971) *Biochim. Biophys. Acta* 241, 697
3 Cohen, L. B., Keynes, R. D. and Hille, B. (1968) *Nature* 218, 438
4 Cohen, L. B., Hille, B. and Keynes, R. D. (1969) *J. Physiol. London* 203, 489
5 Tasaki, I., Watanabe, A., Sandlin, R. and Carnay, L. (1968) *Proc. Natl. Acad. Sci. U.S.* 61, 883
6 Berestovski, G. N., Liberman, E. A., Loonekski, V. and Fank, G. M. (1970) *Biophysika* 15, 62
7 Tasaki, I., (1968) *Nerve Excitation, a Macromolecular Approach*, Charles C. Thomas, Springfield, Ill.
8 Hille, B. (1968) *J. Gen. Physiol.* 51, 221
9 Ehrenstein, G. and Fishman, H. M. (1971) *Nature New Biol.* 233, 16
10 Adelman, Jr, W. J. and Palti, Y. (1969) *J. Gen. Physiol.* 53, 685
11 Hodgkin, A. L. and Huxley, A. F. (1952) *J. Physiol. London* 116, 497
12 Hille, B. (1970) *Prog. Biophys. Mol. Biol.* 21, 1
13 Hoyt, R. C. (1971) *Biophys. J.* 11, 110