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MOLECULAR MECHANISMS OF MEMBRANE IONIC PERMEABILITY CHANGES

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SUMMARY

Simple models were examined suggesting a direct participation of Ca^{2+} in the mechanism of opening and closing the ionic "channels" produced by a shift of membrane potential. It is shown that these models cannot provide a simultaneous quantitative description of two interrelated phenomena: (1) dependance of the peak Na^+ conductance, g_{Na}, on potential E, and (2) the shift of the curve $g_{Na}(E)$ produced by the change of Ca^{2+} concentration in the surrounding solution.

Analysis suggests that the rise of conductance by depolarization is determined by displacement of some charged particles inside the membrane when a mole of particles performs the work $n\delta EF = 3.5EF$ (n, the valence of hypothetical particles, $\delta < 1$; F, the Faraday number).

The physicochemical model of activation of Na^+ conductance of the excitable membrane is studied. The model suggests that there are activating particles inside the membrane which make a transition under the influence of the field connected with the charge displacement. This transition produces the change of Ca^{2+} adsorbed by the membrane from the solution. The model explains the observed form of the curve which shows the dependance of peak Na^+ conductance on membrane potential, the magnitude of this curve shift being produced by the changes of Ca^{2+} in the surrounding solution and the effect of the increase of $[Ca^{2+}]_0$ upon the kinetic effects: the slowing down of the rise of $g_{Na}(t)$ produced by the depolarization and the rise or fall of $g_{Na}(t)$ during repolarization.

INTRODUCTION

The nature of the changes in ionic permeability of an excitable membrane and generation of the biopotential is one of the most important problems of cell biophysics. It is a difficult problem because we do not yet possess any methods to directly observe molecular rearrangements in excited membranes induced by the action of some effector. All information has to be obtained from indirect experiments of the action of different physical and chemical agents on living cells. The action of Ca^{2+} is especially interesting. In our investigations of transport phenomena in biological membranes, we have studied model mechanisms of the ionic permeability of a membrane in a state of rest[1,2]. This paper is concerned with the generation of the action potential, this phenomenon being determined by the change of permeability (*e.g.* ref. 3).

THE DEPENDANCE OF THE PEAK Na^+ CONDUCTANCE ON THE MEMBRANE POTENTIAL

In the experiments with the giant squid's axon performed using the voltage clamp method, HODGKIN AND HUXLEY[4] established an exponential dependance of the peak Na^+ conductance, g_{Na}, on the initial shift of membrane potential. The shift of E to depolarization, equal to 4–6 mV, corresponds to an e-fold increase of g_{Na}. It was suggested that the change of conductance is due to the action of the electric field on some charged particles. If the conductance, g_{Na}, is proportional to the number of these particles shifted from their initial positions[5], then

$$g_{Na} = g_{Na,\max}/[1 + a \exp(-n\delta EF/RT)] \tag{1}$$

where E is the potential of the internal side of the membrane relative to the external potential; constant a does not depend on E; δ is the number representing the portion of the transmembrane potential acting on the charged particle; n, the valence of the particle; and F, the Faraday number. In the region of negative E's g_{Na} is asymptotically expressed by

$$\ln g_{Na} \sim \text{const.} + n\delta \frac{EF}{PT} \tag{2}$$

The slope of the direct line $\ln g_{Na}(E)$ gives the product of two unknown parameters, n and δ. According to ref. 4 $n\delta = 6$. However, this value of $n\delta$ cannot be considered exact. Few points were measured in the region of the linear dependance of $\ln g_{Na}$ from E.

HODGKIN AND HUXLEY[4,5] have not investigated the agreement of the whole experimental curve with Eqn. (1). If this equation is really valid, the parameter $n\delta$ can also be determined from the central part of the curve. At the point $g_{Na} = 0.5\, g_{Na,\max}$ (the inflexion point of the sigmoidal curve) we have

$$\left[\frac{d(g_{Na}/g_{Na,\max})}{d(EF/RT)}\right]_{g_{Na} = 0.5\, g_{Na,\max}} = \frac{n\delta}{4} \tag{3}$$

The dependance, $g_{Na}(E)$, was studied later by other authors on axons of crustacea[6,7] and on myelinated nerve fibers[8]. Analysis of these results with the help of Eqn. (1) shows that this dependance can be satisfactory represented by Eqn. (1).

TABLE I

VALUES OF $n\delta$

The average value of $n\delta$ is 3.5.

Experimental data from:	*Ref. 4*	*Ref. 8*	*Ref. 6*	*Ref. 7*
Calculation according Eqn. 2	6	3.6	3.75	4.5
Calculation according Eqn. 3	2	2	3.3	4.4

Table I contains the values of $n\delta$ calculated by us from experimental data using both methods: the asymptotic formula 2 and formula 3.

Satisfactory agreement between the experimental curves and Eqn. 1 confirms the suggestion that the change in membrane conductivity with the shift of potential is the result of displacement of charged particles by the electric field.

POSSIBLE MECHANISMS OF PARTICIPATION OF THE CHARGED PARTICLES IN CHANGES IN MEMBRANE CONDUCTANCE

According to the carrier hypothesis put forward by HODGKIN *et al.*[9], the initial result of depolarization is the movement of negatively charged particles (carriers with a big negative charge in a complex with Na^+) from the external side of the membrane to the internal side. Experimentation does not confirm this suggestion: the primary ionic current has an opposite direction. HODGKIN AND HUXLEY[5] therefore abandoned this hypothesis and suggested that the charged particles act only as activators and inactivators of the "channels" for Na^+ and K^+ permeability. The hypothesis concerning the possible role of Ca^{2+} in the changes of the sodium and potassium permeability is very attractive.

It is well known[10,11] that changes of Ca^{2+} concentration in the environment strongly influence the excitability of nerve and muscle cells. FRANKENHAEUSER AND HODGKIN[12] used the voltage clamp method for a detailed investigation of the action of Ca^{2+} on ionic currents and membrane conductance with the giant squid's axon. They have shown that a 5-fold change of Ca^{2+} concentration $[Ca^{2+}]_0$ shifts the curves describing the dependance of the Na^+ and K^+ conductance and inactivation from a potential at 10–15 mV. The decrease of $[Ca^{2+}]_0$ shifts these curves to the lower values of E. $[Ca^{2+}]_0$ acts strongly on the rate of "shutting-off" of the Na^+ conductance by repolarization when g_{Na} obtains its peak value. The slowdown of this process by decrease of $[Ca^{2+}]_0$ was much greater than it was expected on the principle that the 5-fold decrease of $[Ca^{2+}]_0$ is approximately equivalent to the magnitude of the depolarization, 15 mV.

THE DIFFICULTIES OF THE CALCIUM HYPOTHESIS

FRANKENHAEUSER AND HODGKIN[12] suggested a possible explanation of the action of $[Ca^{2+}]_0$ on conductance and inactivation. Ca^{2+} can be adsorbed at the external side of the membrane and produce an electric field additive to the transmembrane potential. This explains the parallelism between the changes of $[Ca^{2+}]_0$ and the changes of membrane potential[13], but the marked action of $[Ca^{2+}]_0$ on the rate of decrease of Na^- conductance after repolarization remains a mystery. According to another hypothesis[12], Ca^{2+} play the role of particles blocking the ionic channels. In the state of rest at negative E's, Ca^{2+} is held in these channels by forces of the electric field. Depolarization of the membrane or a decrease in $[Ca^{2+}]_0$ increase the probability of channels being free and the conductances increase. An opposite change of conductance occurs if the membrane is hyperpolarized or if $[Ca^{2+}]_0$ is increased. The ratio of the number of channels closed by Ca^{2+}, P, and of the number of open channels, $(1-P)$, must be proportional to $[Ca]_0$. Then

$$P/(1-P) = b[Ca^{2+}]_0 \exp(-2\delta EF/RT) \qquad (4)$$

where δ is the portion of potential moving Ca^{2+} from the external fluid to the place where they close the channel. If this place is located at the middle of the membrane, $\delta = 0.5$, and if it is near the internal side, $\delta \simeq 1.0$, then b is a constant. According to Eqn. (4), the e-fold change of $[Ca^{2+}]_0$ is equivalent to the change of E not lower than 12.5 mV (if $\delta = 1.0$). However, experiments[6–8, 12] show that this change is 6–9 mV. Another difficulty arises if we consider the value of $n\delta \simeq 4.0$. We have $\delta \leq 1$, therefore $n \gtrsim 4$, but the charge of Ca^{2+} is only 2. It can be suggested that every channel is blocked by several Ca^{2+}. However, such a suggestion creates another contradiction. If m Ca^{2+} block each ionic channel simultaneously, Eqn. (4) becomes

$$P/(1-P) = b[Ca^{2+}]_0^m \exp(-2m\delta EF/RT) \tag{5}$$

If $g_{Na} \sim (1-P)$, then

$$g_{Na} = g_{Na,\max}/[1 + b[Ca^{2+}]_0^m \exp(-2m\delta EF/RT)] \tag{6}$$

This formula coincides with Eqn. (1) if $n = 2m$ and $a = b[Ca^{2+}]_0^m$. The curve (6) shifts along axis E if $[Ca^{2+}]_0$ changes. Equal values of g_{Na} correspond to the condition

$$\ln[Ca^{2+}]_0 - 2\delta EF/RT = \text{const.} \tag{7}$$

The shift of the curve $g_{Na}(E)$ with the change of $[Ca^{2+}]_0$ remains the same as in the first kind of calcium hypothesis, *i.e.* suggesting that the channel is blocked by one Ca^{2+}. This shift is much bigger than the observed one.

TASAKI[14] suggested that the dependance of membrane conductance from degree of depolarization can be explained by the theory of cooperative processes, using the method of molecular field (see, *e.g.* ref. 15). CHANGEUX *et al.*[16] described in this way some membrane properties. Let us therefore see how this method can be applied to the statistical model of an excitable membrane.

A membrane is considered as a system of periodically arranged sites. Each site can exist in two states: conductable and non-conductable. The conductance of the membrane is proportional to the number of sites in the first state. The energy of interaction of the neighbouring sites is equal to W_{ii} if they are in the same state, and to W_{ij} if their states are different. Let $W = W_{ii} - W_{ij}$. Each site can bind an ion of valence n, which crosses the difference of potentials E moving from the solution into the membrane. The binding changes the energy of site at $U + n\delta EF$ (per mole). The ligand (*e.g.* Ca^{2+}) makes the site non-conductable. The molecular field approximation describes the system by equation of lattice gas (see, *e.g.* ref. 17). We get

$$\frac{P}{1-P} \exp\left[\frac{\nu W(2P-1)}{RT}\right] = b[Ca^{2+}]_0 \exp\left(-\frac{n\delta EF}{RT}\right) \tag{8}$$

where ν is the coordination number. For a non-cooperative system $W = 0$, and Eqn. (8) is equivalent to Eqn. (5) if $m = 1$[*].

The derivative of (8) gives at the point $P = 0.5$

$$\left[\frac{dP}{d(EF/RT)}\right]_{P=0.5} = -\frac{n\delta}{2(2-\beta)} \tag{9}$$

where $\beta \equiv \nu W/RT$ — parameter of cooperativity. As $g_{Na} \sim (1-P)$,

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[*] TASAKI[14] investigated the volt-ampere characteristics of excitable membrane. He introduced the quantity α, the ratio of the membrane surface in the active state and the whole surface. This quantity is equivalent to $1-P$. From the curve α (E) presented in ref. 14 we got $[d\alpha/d(EF/RT)]_{\alpha=0.5} = 1.1$.

$$\left[\frac{\mathrm{d}(g_{\mathrm{Na}}/g_{\mathrm{Na,\,max}})}{\mathrm{d}(EF/RT)}\right]_{g_{\mathrm{Na}} = 0.5\, g_{\mathrm{Na,\,max}}} = \frac{n\delta}{2(2-\beta)} \tag{10}$$

Let us compare this value with Eqn. (3). In the absence of cooperativity ($\beta = 0$) both formulae are equivalent. Experiment gives the tangent of the slope angle in the point $g_{\mathrm{Na}} = 0.5\, g_{\mathrm{Na,max}}$ of the order 1 (refs. 4, 6–8). Therefore, from Eqn. (3) it follows that $n \simeq 4$. Eqn. (10) can give the same tangent of angle at any $n < 4$ with suitable value $\beta > 0$. Hence the slope can be explained if $n = 2$. The shift of this curve with the change of $[\mathrm{Ca}^{2+}]_0$ can be estimated in the following way. Similar to Eqn. (7) the condition for the shift is

$$\ln\, [\mathrm{Ca}^{2+}]_0 - n\delta EF/RT = \text{const.} \tag{11}$$

And if $[\mathrm{Ca}^{2+}]_0$ changes e-fold, we get

$$E = 25/n\delta\ (\mathrm{mV}) \tag{12}$$

As we have shown, the form of the curve $g_{\mathrm{Na}}(E)$ can be explained by this model even if $n\delta \leq 2$. However, according to Eqn. (12), ΔE agrees with the experimental value only if $n\delta \simeq 3.5$. Thus the theory of cooperative processes can explain the high steepness of the curve $g_{\mathrm{Na}}(E)$, but it cannot explain the shifts of $g_{\mathrm{Na}}(E)$ produced by the changes in $[\mathrm{Ca}^{2+}]_0$.

Examining all these models we came to conclusion that the simultaneous explanation of both interdependent effects of the form of the curve $g_{\mathrm{Na}}(E)$ and the shift of this curve following the change of $[\mathrm{Ca}^{2+}]_0$ in the surrounding solution needs another model. The strong increase of conductance is due to the movement of the charge $n\delta \geqslant 4$ inside the membrane and this movement must be connected with desorption of one Ca^{2+}. If the charge performs the work $n\delta EF$ in the field (where $n\delta = 3.5$), then, according to Eqn. (10), we must take $\beta \simeq 0$ for agreement with experiment. It means that the single conductance channels practically do not interact.

Recently CHISMADJEV[18] studied the same problem qualitatively using the idea of cooperativity to explain the experimental curves. They gave a phenomenological calculation. The success of this attempt depends on the quantitative evaluation of parameters. As it was said already, we obtained $\beta = 0$.

It has to be emphasized that any modification of the calcium hypothesis can explain only one side of the action of field on the excitable membrane, namely the activation of the ionic conductances. Inactivation is not treated here. This means that some other charged particles displaced by the field have to be taken into account.

THE MODEL OF ACTIVATION

The activation process must be described by the following set of reactions:

$$\mathrm{XCa} \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} \mathrm{X} \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} \mathrm{Y} \tag{13}$$

where X and Y denote two states of the activating molecule differing by distribution of charge in the membrane. The transition of the molecule to the state Y corresponds to the transition of the surrounding part of the membrane in the high-conductive state. Let us denote the numbers of molecules in states X, Y, XCa by x, y, s, respectively. Then the Na+ conductance of the membrane can be expressed by

$$g_{Na} = ky \qquad (14)$$

where k is the proportionality coefficient. The study of the changes of conductance means the study of dependance of y on time. Let

$$K_i = k_i'/k_i \quad (i = 1,2) \qquad (15)$$

According to the suggestion of the displacement of charge performing the work $n\delta EF = 3.5\ EF$ in the transition X $\rightleftharpoons$ Y, we can write

$$K_2 = q \exp(-n\delta EF/RT) \qquad (16)$$

where factor q does not depend on the field.

The system of kinetic equations describing the scheme of reactions (13) is

$$\begin{aligned} \dot{y} &= k_2x - k_2'y \\ \dot{s} &= k_1'[\mathrm{Ca}^{2+}]_0x - k_1s \\ x + y + s &= N \end{aligned} \qquad (17)$$

where N is the total number of activating molecules.

Analysis of the experimental data shows that the peak Na+ conductance corresponds to the value y near the equilibrium value y_∞. Thus

$$g_{Na} \simeq ky_\infty \qquad (18)$$

In the steady state $\dot{y} = \dot{s} = 0$. We get

$$y_\infty = \frac{N}{1 + K_2 + K_1K_2[\mathrm{Ca}^{2+}]_0} \qquad (19)$$

and from Eqns. (18), (19) and (16) it follows that

$$g_{Na} = g_{Na,max}/[1 + (1 + [\mathrm{Ca}^{2+}]_0K_1)q \exp(-n\delta EF/RT)] \qquad (20)$$

where $g_{Na,max} = kN$.

If the value K_1 is big enough (it corresponds to the shift of reaction XCa $\rightleftharpoons$ X to the left), we have

$$[\mathrm{Ca}^{2+}]_0K_1 \gg 1 \qquad (21)$$

This condition corresponds to the introduction of the state XCa as a "reservoir" of activating particles introduced by HODGKIN *et al.*[9]. Taking into account Eqn. (21) we get the formula (1) treated earlier, where $a = q[\mathrm{Ca}^{2+}]_0K_1$. If $n\delta = 3.5$ formula (20) described the curve $g_{Na}(E)$ and gives the quantitative explanation of its shift with the change of $[\mathrm{Ca}^{2+}]_0$.

As well as works where the influence of $[\mathrm{Ca}^{2+}]_0$ on the membrane conductance was studied[12], there are works devoted to studying the action of other cations[6-8]. The e-fold change of concentration of many bivalent cations produces the same shift of $g_{Na}(E)$ for 6–8 mV. These data seem to be natural from the point of view of the suggested model as the role of Ca2+ can also be played by other bivalent ions. The constant K_1 can vary, but if it remains big enough the shift does not depend on K_1.

HILLE[8] studied the action of pH on $g_{Na}(E)$. If the pH < 5.5, the shift $\Delta E_H = 13.5$ mV, and if the pH > 5.5, $\Delta E_H = 1.3$ mV (*e*-fold change of H^+ concentration). Others cations were not studied in such a broad range of concentrations $(1 \cdot 10^{-4}$–$1.10^{-10})$.

Our analysis shows that every conductance channel adsorbs one bivalent ion. If H^+ perform the same function, it can be suggested that their number is double in comparison with Ca^{2+}. Substituting $[H^+]_0$ for $[Ca^{2+}]_0$ in Eqn. (20) we get the condition

$$(1 + [H^+]^2{}_0K_1) \exp(-n\delta EF/RT) = \text{const.} \tag{22}$$

or

$$\ln(1 + [H^+]^2{}_0K_1) - n\delta EF/RT = \text{const.} \tag{23}$$

Let us consider two limiting cases. If K_1 or $[H^+]_0$ are so big that

$$[H^+]^2{}_0K_1 \gg 1 \tag{24}$$

we get

$$2 \ln[H^+]_0 - n\delta EF/RT = \text{const.} \tag{25}$$

In this case the *e*-fold increase of $[H^+]_0$ is equivalent to a decrease of E equal to

$$\Delta E_H = \frac{2RT}{n\delta EF} \tag{26}$$

As $n\delta \cong 3.5$, $\Delta E \cong 14$ mV in good agreement with the results of HILLE[8] for the region pH < 5.5.

In the region of very small $[H^+]_0$, if

$$[H^+]^2{}_0K_1 \ll 1 \tag{27}$$

we get

$$[H^+]^2{}_0K_1 - nEF/RT = \text{const.} \tag{28}$$

This equation does not give a linear dependance of E on $\ln[H^+]_0$. If the tangent of slope angle of the line $E(\ln[H^+]_0)$ is equal to $2RT/n\delta F$ according to Eqn. (25), then if Eqns. (27) and (28) are valid for the curve $E(\ln[H^+]_0)$ the tangent becomes $2RT[H^+]_0{}^2K_1/n\delta F$. Using Eqn. (27) we see that the slope becomes much smaller. We cannot estimate it quantitatively as it depends on the unknown constant K_1. Fig. 1 shows the dependance of E on $-\ln[H^+]_0$. The solid lines correspond to the studied asymptotic portions of the curve, the dotted lines to their continuation and the dashed curve represents the region of junction of asymptotic portions. The dependance ex-

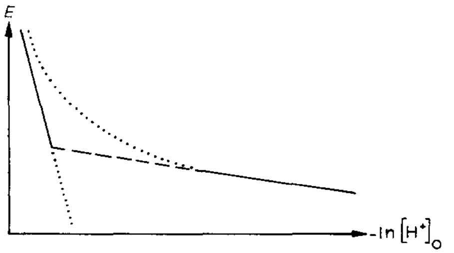

Fig. 1. Dependence of E on $-\ln[H^+]_0$ when the conductance g_{Na} is constant. Solid lines correspond to the studied asymptotic portion of the curve, the dotted lines to their continuation and the dashed curve represents the region of junction of asymptotic portions.

perimentally observed by HILLE[8] has the same form (ref. 8, Fig. 6). The left part of this curve is explained quantitatively by the theory presented here.

The same form has to be expected for the corresponding dependance in the case of bivalent ions.

Another interesting regularity observed by HILLE[8] in the experiments with varying pH is the change of the maximal value of the peak conductance $g_{\mathrm{Na,max}}$. The dependance of $g_{\mathrm{Na,max}}$ from pH has the form of the dissociation curve of the weak acid with $\mathrm{p}K = 5.2$. Such an effect is produced by La^{3+} (ref. 7); this ion decreases $g_{\mathrm{Na,max}}$ strongly. As $g_{\mathrm{Na,max}} = kN$, the decrease of this value can be explained suggesting the formation of stable complexes with the charges of activating molecules and the corresponding decrease of N.

DESCRIPTION OF KINETIC PHENOMENA

The suggested scheme allows to treat kinetic phenomena. System (17) involves two linear differential equations therefore function $y(t)$ must have the form

$$y = A + Be^{r_1 t} + Ce^{r_2 t} \tag{29}$$

The constants A,B,C,r_1,r_2 depend on the parameters of the system k_i, k_i', N, $[\mathrm{Ca}^{2+}]_0$. It can be shown that the curve $y(t)$ possesses one inclination point. Really such form was observed experimentally for the curves $g_{\mathrm{Na}}(t)$ (ref. 12, Fig. 4). Choosing the constants in the suitable way it is possible to obtain the coincidence of the theoretical and experimental curve. However, we shall not perform these calculations but we shall try to examine analytically the influence of $[\mathrm{Ca}^{2+}]_0$ on kinetic curves. Let us consider the case k_1, $k_1' \ll k_2$, k_2'. If the rate of the second reaction is much higher than of the first one, the equilibrium between X and Y will be established faster than between X and XCa. In such an adiabatic approximation the system (17) gets the form

$$\begin{aligned} \dot{s} &= -k_1 s + k_1'[\mathrm{Ca}^{2+}]_0 x \\ k_2 x &= k'_2 y \\ x + y + s &= N \end{aligned} \tag{30}$$

We get from (30)

$$\dot{y} = (k_1 s - k'_1 K_2 [\mathrm{Ca}^{2+}]_0 y)/(K_2 + 1) \tag{31}$$

If at the beginning of observation the quantity y is already near to its equilibrium value corresponding to the depolarization, then according (19) $y_\infty \to N$ at big E. In such a case the initial conditions for Eqn. (31) have the form $y_0 \cong N$, $s_0 \cong 0$. Eqn. (31) gives

$$\dot{y}_0 \cong -k_1 N [\mathrm{Ca}^{2+}]_0 K_2/(K_2 + 1) \tag{32}$$

This formula must describe the fall of g_{Na} if the depolarization is switched off at the moment of rise of Na^+ current to its peak value. FRANKENHAEUSER AND HODGKIN[12] observed that the rate of fall of g_{Na} depends strongly on $[\mathrm{Ca}^{2+}]_0$: the decrease of $[\mathrm{Ca}^{2+}]_0$ from 112 to 22 mM slows down the fall of conductance 5-fold, from 112 to 4.4 mM 10–15-fold. In the last case the effect is complicated as the rate of fall of g_{Na} becomes equal to the rate of natural inactivation. Therefore the authors suggest that the slow down in this case could be more than 15-fold. The experiment also shows that the influence of the decrease of $[\mathrm{Ca}^{2+}]_0$ upon the rate of fall of conductance is considerably greater than that according to the rule: "5-fold decrease of $[Ca^{2+}]_0$ is equivalent to the depolarization of the magnitude 10–20 mV" established for other effects as, *e.g.* the influence of $[Ca^{2+}]_0$ on the curve $g_{Na}(E)$. The hypothesis of HUXLEY explaining the influence of $[Ca^{2+}]_0$ on the membrane conductance was abandoned precisely because of the impossibility to explain these kinetic effects[12].

Eqn. (32) obtained on the base of our physicochemical model explains both these effects. Firstly this formula shows that the rate of fall of g_{Na} has to be proportional to $[Ca^{2+}]_0$, *i.e.* the decrease of $[Ca^{2+}]_0$ from 112 to 22 mM must indeed produce the 5-fold decrease of the rate and the decrease from 112 to 4.4 mM the 25-fold decrease of the rate. Secondly, the formula (32) shows that if the decrease of $[Ca^{2+}]_0$ is compensated by the shift of potential in such a way that the product $[Ca^{2+}]_0K_2$ remains constant, the decrease of the rate of fall of g_{Na} has to be observed as K_2 enters the denominator too.

Let us examine now the influence of Ca^{2+} on the kinetics of rise of g_{Na} if depolarization is switched on. The initial condition $y_0 \cong 0$ corresponds to this process and the solution of Eqn. (31) gets the form

$$y = N\frac{1 - \exp\left[-(1 + K_2 + K_1K_2[Ca^{2+}]_0)k_1t/(1 + K_2)\right]}{1 + K_2 + K_1K_2[Ca^{2+}]_0} \tag{33}$$

The rate of growth of $y(t)$ is

$$\dot{y} = k_1N\frac{\exp\left[-(1 + K_2 + K_1K_2[Ca^{2+}]_0)k_1t/(1 + K_2)\right]}{1 + K_2} \tag{34}$$

We get a simple exponential rise instead of the curve with an inclination which was obtained solving the exact system (17). This follows from the adiabatic approximation. But the formulae (33) and (34) explain the smaller slope of the curve $g_{Na}(t)$ obtained in the experiments with bigger concentrations of Ca^{2+}.

If the shift of potential is big, $K_2 \to 0$ and Eqn. (33) transforms into

$$y(t) \simeq N(1 - e^{-k_1t}) \tag{35}$$

It follows from (35) that the lower the probability of dissociation of the cation from the activating particle, the slower the transition to the peak Na^+ current has to be. This current does not depend on the constants k_i in this case. Hence it is possible to treat some features of the action of Ni^{2+} on the membrane conductance. It is known (*e.g.* ref. 19) that these ions slow down the activation process considerably. Simultaneously the data concerning the action of Ni^{2+} on the shift of $g_{Na}(E)$ (ref. 6) and on the hyperpolarization responses[20] testify in favor of the stronger binding of Ni^{2+} by membrane phospholipids, *i.e.* the constant k_i for Ni^{2+} is smaller than it is for Ca^{2+}.

CONCLUSION

The analysis of experimental data shows that the change of the peak sodium conductance of membrane due to the change of potential is satisfactorily represented by Eqn. (1) with parameter $n\delta$ equal to 3.5. The suggestion that the increase of conductance can be caused by removal of one Ca^{2+} from the conducting channel cannot explain the form of the curve $g_{Na}(E)$ and its shift due to change of $[Ca^{2+}]_0$.

The hypothesis suggesting the closing of channel by several Ca^{2+} describes the form of the curve $g_{Na}(E)$ but not its shift.

The model which takes into account the cooperative processes in membranes explains the experimentally observed slope of the curve $g_{Na}(E)$ without the suggestion that $n\delta = 3.5$. According to the cooperative model $n\delta$ can be less than 2.0 if the parameter of interaction of the neighbouring sites is big enough. It means that the activating particles displaced by the field can be a bivalent ion. However, the quantitative description of the shift of the curve in this model also demands the value of n to be approximately equal to 3.5.

This examination of different kinds of "calcium hypothesis" suggests that the increase of conductance produced by the shift of potential is determined by displacement of some charged particles performing the work $n\delta EF = 3.5EF$. These particles are not Ca^{2+}, but their displacement is probably linked with adsorption and desorption of Ca^{2-}.

Taking into account these conclusions a simple physicochemical model for the activation process, is proposed, explaining both the form of curve "peak Na^+ conductance–membrane potential", and the value of shift of this curve along the axis of the electric force due to the change of concentration of Ca^{2+} and other ions in surrounding solution. The same model explains also the influence of $[Ca^{2+}]_0$ on kinetic effects — the slowing down of the growth of g_{Na} due to depolarization and the increase of the fall of g_{Na} due to repolarization.

The process of inactivation is not considered in this paper. The generalization of the model described here is presented in another work[21]. Suggesting that the activating particle in state Y can undergo some physicochemical transformation which is not connected with redistribution of charges, it is possible to obtain the curve of steady-state inactivation in good agreement with experiment, and to get the quantitative description of the shift of this curve called forth by the change of $[Ca^{2+}]$. This theory does not involve any additional parameters except $n\delta = 3.5$.

It can be suggested that the change of K^+-conductance is determined by the similar mechanism[12,22].

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