Ion Regulation of the Kinetics of Potential-Dependent Potassium Channels

O.V. GRISHCHENKO¹, V.N. KHARKYANEN¹, N.I. KONONENKO² and G.E. WEINREB¹

¹*Institute for Physics, Division for Physics of Biological Systems, Kiev, Ukraine;* ²*A.A. Bogomoletz Institute of Physiology, Kiev, Ukraine*

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Abstract. We apply a theoretical approach developed earlier. The interaction of ions that permeate a channel with slowly relaxing charged channel-forming groups (ion-conformational interaction – ICI) is addressed by this approach. One can describe the ion concentration influence (ion regulation) on channel functioning in this manner. A patch-clamp method in a ‘whole-cell’ configuration is used to study the ICI. For this purpose the influence of an external concentration of potassium ions on the potential-dependent potassium current (I_A) in the external membrane of GH₃ cells was studied. The increase of $[K^+]_{out}$ from 5 mM to 100 mM causes a non-monotonous shift of current-voltage dependencies. The dependence of both an activation time constant (τ_n) and a steady-state activation (n_∞) on $[K^+]_{out}$ have a minimum and maximum respectively. The analysis of the results suggests that the observed effects are caused by ICI. A physical model is developed to describe the dependence of the potassium channel kinetics on the external concentration of the ions and the membrane potential. The ‘deformation’ of the closed state of the gate and the corresponding energy shifts cause the observed non-monotonous dependencies due to ICI. Thus, the general theoretical approach has an experimental confirmation and is applied to concrete examples. Formulas for concentrational dependencies of the channel kinetics are given for practical uses.

Key words: Potential-dependent ion channels, Channel kinetics, Concentrational dependencies, Ion-conformational interaction (ICI)

1. Introduction

Ion channels of biological membranes are protein macromolecules located in the lipid bilayer and form pores there. Analysis has shown that an interaction between a limited number of some amino acid groups and permeant ions determines such channel characteristics as ion selectivity, permeability and conductivity [6, 12, 14, 23, 28]. The dependence of the probability of the channel open state on voltage shifts under the influence of the following factors:

- change of the type of a permeant ion [26];
- change of ion concentrations in potassium [31] and calcium [10] channels;
- change of the gating charges [21].

If only the external electric field (the membrane potential) acts directly on the voltage sensor and is the only determinant controlling the channel kinetics, then there is no acceptable explanation for the cited experiments. That is why it is

necessary to take into consideration the influence of ions on the sensor. Indeed, the membrane potential causes electric fields in the channel of about 10^5 V/cm. But an ion in its immediate environment causes the fields of about 10^9 V/cm. If the relaxation time of the structural channel groups interacting with permeant ions is so short that molecular groups shifted by this action have enough time to relax before the next ion comes in, then every passing ion will interact with the same conformation of the channel. In such a case no change in ion concentration can significantly influence the channel functioning. However, if the relaxation time of the structural channel groups interacting with permeant ions is large enough, then, having been shifted by single ion movements, the groups cannot return to their initial position before the next ion enters. In this case the shifts caused by single ions are summed and the average number of ions in the channel will determine the position of molecular groups interacting with ions. Such changes in the position of molecular groups in the channel can significantly influence ion flux characteristics, including kinetics. Thus, a physical variable determining ion-conformational interaction (ICI) is the mean number of ions in the channel (we call it channel occupancy N).

It was shown earlier that ICI causes a shift of the dependencies of the open state probability on the membrane voltage [29], as well as the appearance of additional levels of conductivity [4, 14, 30] under change of ion concentrations. We suggest that the influence of permeant ions on ion channel function must have many manifestations. Their experimental study and theoretical modelling are the aim of this paper.

Some properties of transformed GH₃ cells make them a suitable model for the study of the effects of voltage-dependent potassium currents. These cells possess only three types of outward potassium currents: the rapidly inactivating current I_A (Kv1.4 channels), the slowly activating and the inactivating current (Kv1.5 channels) and the $[Ca^{2+}]_{in}$ -activated channels responsible for $I_{K(Ca)}$ potassium currents [24]. Being spherical and of approximately the same size makes GH₃ cells a suitable subject for electro-physiological research.

As a rule, existing theoretical models describe ion channel kinetics without taking into consideration the influence of permeant ions on channel functioning. P. Lauger was one of the first to report various rates of transition from the closed to the open state depending on ion presence in the channel [16]. However, Lauger considered the states of the channel to be unchangeable and their number to be a discrete set constant while active and under different experimental conditions. Our data confirm that such an approach has to be modified and we have to take into account the changes of the states themselves under external conditions. We develop a corresponding physical approach that adequately describes the experimental data.

2. Experiment

2.1. METHODS

To conduct the experiments we used GH₃ cells line isolated from rat pituitary tumour. They were obtained from the cell culture collection at the Institute of Cytology (St Petersburg). They were cultured as a monolayer culture in plastic dishes (75 mm²) and/or in Petri dishes (30 mm). The cells were plated every 3 to 4 days in the 1:2 ratio. GH₃ cells were cultured in RPMI medium 1640 (Sigma) supplemented with 10% fetal bovine serum at 37 °C in humidified atmosphere containing 5% CO₂. Cells were plated at a density of $1 - 2 \times 10^5$ cells per Petri dish with glass coverslips and used one to four days after plating.

Before each electro-physiological experiment the culture medium in Petri dishes was replaced with a corresponding bath solution. We replaced the bath solution through an application pipette that was brought at approximately 100 μ m to the investigated cell. The 'patch-clamp' technique in 'whole-cell' configuration was used in our experiments [9]. A patch-clamp recording electrode was filled with the bath solution containing (in mM) KCl – 40, KF – 100, Tris-HCl (pH 7.4) – 70, EGTA – 1. The basic bath solution had the following constitution (in mM): KCl – 5, CaCl₂ – 1, MgCl₂ – 2, Tris-HCl (pH 7.4) – 150, glucose – 5. The substitution of the corresponding Tris-HCl quantity into the equivalent KCl quantity changed the external potassium ion concentration.

In all the experiments the membrane potential was held at –90 mV; in most cases the currents were registered once a minute and the duration of the testing potential was 20 ms. We used the procedure of digital subtraction (P/4) together with analogous subtraction of both the capacity and leakage currents. Only cells with a small slowly inactivating component were used (i.e., peak current of the slowly inactivating component was at least in ten times smaller than the peak of I_A).

2.2. RESULTS

Figure 1 shows oscillograms of a voltage-dependent transient potassium current in GH₃ cells and its current-voltage relationships (CVR). The activation threshold for the K⁺-current ranged from –50 to –40 mV.

Figure 2 shows peak current dependencies on the potential difference $\varphi - \varphi_K$ (φ is the membrane potential and φ_K is the equilibrium potential for potassium) for different concentrations of K⁺ ions in the outer solution. A shift of the curves with an increase of $[K^+]_{out}$ concentration from 5 mM to 100 mM was observed.

We calculated the number of activation particles (it was found to be 4) as well as the values of the activation time constant (τ_n) of the potassium current on the basis of I_K traces using the Hodgkin-Huxley formalism [11]. The values of the steady-state activation (n_∞) of the K⁺-current were also found. According to the classical model [11] τ_n and n_∞ are a function only of the membrane potential and do not depend on the value of the ion current. Nevertheless, as we have already

Figure 1. Activation of depolarisation-activated outward transient potassium current (I_A) in GH₃ cells. Depolarisations from a holding potential of -90 mV to potentials between -20 and $+60$ mV were applied in 20 mV increments.

Figure 2. Peak current dependencies on $\varphi - \varphi_K$.

mentioned above, a shift in the dependence of n_∞ on the membrane potential towards a shift of the reversal potential has been detected [27, 31, 33]. In our case the registered shift was of a non-monotonic character (Figure 3a). With an increase of the $[K^+]_{out}$ from 10 to 20 mM the curve is offset to lower potentials, but with the external potassium concentration 30 mM the same curve is offset backwards to higher potentials. A similar effect has been observed in the dependencies of τ_n on the membrane potential φ (Figure 3b).

To understand this phenomenon we considered the kinetic scheme for transitions from open to closed state and reverse

Figure 3. Dependencies of the steady-state activation n_∞ (a) and activation time constant τ_n (b) on membrane potential at different concentrations of $[K^+]_{out}$.

$$\begin{array}{c} \beta \\ \mathbf{0} \rightleftharpoons \mathbf{c}. \\ \alpha \end{array} \quad (1)$$

The transition rates α and β are related to τ_n and n_∞ by the following well-known relationship

$$\tau_n = [\alpha + \beta]^{-1} \quad (2)$$

$$n_\infty = \frac{\alpha}{\alpha + \beta}. \quad (3)$$

(As we mentioned above, the channel kinetics is determined by fourth order of $n(t)$.)

We developed a physical model for the channel kinetics taking into account interactions between potassium ions and channel charged groups (see Section 3: Theory). The notion of the important role of the ion-conformational interaction in channel functioning was developed earlier in [4, 14, 29, 30] by some of the present authors. Here we obtain the dependency of the kinetic parameters τ_n and n_∞ on the external concentration $[K^+]_{out}$. Figure 4a shows the computed dependencies of the steady-state activation n_∞ on the external potassium concentration. Figure 4b shows the same for the activation time constant τ_n . The curves are the results of modelling and the circles correspond to experimental data.

Figure 4. Experimental data of kinetic characteristics as a function of $[K^+]_{out}$ (circles) and the corresponding results of theoretical modelling according to Equations (14). Model parameters: $\alpha_0 = 0.839/\text{ms}$, $\beta_0 = 0.14/\text{ms}$, $c_1 = -0.017/\text{mV}$, $c_3 = 0.022/\text{mV}$, $\gamma = 6$, $N_0 = 0.476$, $\kappa = 4.197$, $\Omega = 15 \times 10^6$ mM.

Figure 5. Double-well conformational potential determining the kinetics of functioning of the ion channel: the probabilities of realisation of state, life time and frequency of transitions are determined by the values of the potential at the extrema – $U(x_1)$, $U(x_2)$ and $U(x_3)$ (in our case x_1 corresponds to the open state; x_3 corresponds to the closed state). Change in the membrane voltage φ leads to new relations between these values and hence to a change in the kinetics of the channel. The so-called gate processes occur by this principle and according to the Hodgkin-Huxley theory [11].

3. Theory

3.1. GENERAL SPECULATIONS

Let us consider with the functioning of the channel. The Hodgkin-Huxley model assumes that several gates are responsible for opening-closing of a channel and determine the ion channel kinetics. Figure 5 demonstrates the functioning of such a gate (compare to scheme (1)). Such gates can also be found in two states: for opening and closing the channel. But they can also be found in any state that corresponds to an certain value of a generalised conformation variable x . (This variable can describe the channel effective diameter at a certain point, position of some charged channel group, some angle or molecular size, etc.) Every state is characterised by an energy which is a function of x . This energy we call the conformational potential and indicate it as $U(x)$. The minima determine stable states: x_1 corresponds to the open state and x_3 to the closed; x_2 corresponds to the position of the potential maximum determining the energy necessary for the transition from one minimum to another. The effective potential $U(x)$ completely determines the kinetics of the transitions, which have a thermo-activational character and can take place when the energy of the particle exceeds the difference between the energy of the particle at the barrier x_2 and in the corresponding potential well x_i . The transition rates α and β are determined by the following equations:

$$\begin{aligned}\alpha &\sim \exp \left[\frac{U(x_3) - U(x_2)}{kT} \right] \\ \beta &\sim \exp \left[\frac{U(x_1) - U(x_2)}{kT} \right]\end{aligned}\quad (4)$$

(k is Boltzmann's constant and T is an absolute temperature).

To describe an ICI and its influence on channel functioning we divide $U(x)$ into two terms of the sum:

$$U(x) = H(x) + V(x) \quad (5)$$

where

$H(x)$ is the energy value of a gate without ions in the channel. It depends on the membrane voltage but not on the concentration of permeant ions;

$V(x)$ is the energy value of an ICI. It includes an occupancy of the channel by ions N and in general cases depends on both the membrane voltage and ion concentrations in bath solutions.

Equations (4) determine the dependencies of the kinetics of gate processes on the mutual positions of the conformational potential $U(x)$ extrema. The channel potential $H(x)$ near the open (x_1) and closed (x_3) states we can always represent as parabolas:

$$H(x) = a_i x^2 + b_i x + kT c_i \varphi,$$

Figure 6. Dependencies of transition rates α and β on $[K^+]_{out}$.

$$x_i - \Delta_i \leq x \leq x_i + \Delta_i, \quad (6)$$

where $i = 1$ or 3 , φ is the membrane potential and a_i , b_i and c_i are fixed structure parameters of the channel, Δ_i are positive values determining deviations from the minima x_i where the approximation (6) takes place. Here we have introduced kT at the term $c_i\varphi$ for convenience.

3.2. THE MODEL

The observed dependencies, in Figures 3 and 4, of kinetic parameters on $[K^+]_{out}$ are caused by the ICI that is described by term $V(x)$ in Equation (5). To analyse this term we examine transition rates α and β . Figures 6a and 6b show the experimental dependencies of α and β on the $[K^+]_{out}$ that we can easily calculate using Equations (2–3). Thus, we observed noticeable non-monotonic dependencies of kinetic parameters on $[K^+]_{out}$ achieving a saturation at concentrations more than 30 mM. The figures demonstrate that the dependencies of α on the potassium concentration are more clearly displayed than β -dependencies that remain practically stable upon variation of potassium concentrations. From Equations (4) one can see that this means that the difference $U(x_1) - U(x_2)$ is unchangeable whereas the difference $U(x_3) - U(x_2)$ is influenced by $[K^+]_{out}$. Thus we can conclude that the closed state (x_3) happens to be sensitive to the external potassium concentration at a constant unstable state (x_2). At the same time the open state (x_1) is not affected by variations of $[K^+]_{out}$ at all. On the other hand it is necessary to point out that the extremum of the dependence of α on $[K^+]_{out}$ is not shifted by the membrane potential variations, though it might be expected that ion occupancy of the channel should determine ICI, and should depend on both ion concentrations and the membrane potential.

Thus, analysis of the experimental data, leads us to conclude that the observed effects are caused by the ‘deformation’ of the closed state of gates and that the membrane potential does not influence ICI. This could mean that ion-conformational interaction is determined by the occupancy of the channel at the position (binding site) close to the outer solution and there is no drop of membrane potential. Due to the faster exchange with the bath solution in comparison to the exchange with the channel solution the channel occupancy at the mentioned binding site is determined basically by the external potassium concentration but not by the potential and/or internal concentration.

Thus, taking into consideration the fact that $[K^+]_{\text{out}}$ does not change the transition rate β , let us consider neglecting the ICI term $V(x)$ at the position corresponding to the open state (x_1):

$$V(x) = 0,$$

$$x_1 - \Delta_1 \leq x \leq x_1 + \Delta_1. \quad (7)$$

To make it simpler we further consider the x_2 position and the corresponding $U(x)$ value to be fixed ($U(x_2) = \text{const}$). Thus we consider only the potential determining the closed state (x_3) to be sensitive to the $[K^+]_{\text{out}}$ variations. This sensitivity is due to the ICI term $V(x)$. Once again we refer to the simplest case when the ion conformational force is constant, that is

$$\frac{dV(x)}{dx} = \eta N, \quad (8)$$

where N is an ion occupancy and is the main physical value determining the ICI. η is the force acting when the ion is permanently located in the channel ($N = 1$). It is obvious that ICI is proportional to the number of ions taking part in the interaction. Integrating expression (8) over x we obtain the potential $V(x)$

$$V(x) = \eta N x + \mu N,$$

$$x_3 - \Delta_3 \leq x \leq x_3 + \Delta_3, \quad (9)$$

where the integration constant is proportional with coefficient μ to N . In this case both the ion-conformational force, as well energy, are equal to zero when the binding site is empty ($N = 0$).

Thus, the conformational potential $U(x)$ can be described in the following way: at the open state

$$U(x) = a_1 x^2 + b_1 x + kT c_1 \varphi,$$

$$x_1 - \Delta_1 \leq x \leq x_1 + \Delta_1. \quad (10.a)$$

and at the closed state

$$U(x) = a_3x^2 + (b_3 + \eta N)x + kTc_3\varphi + \mu N,$$

$$x_3 - \Delta_3 \leq x \leq x_3 + \Delta_3. \quad (10.b)$$

From the condition determining the positions of the minima x_1 and x_3

$$\frac{dU(x)}{dx} = 0 \quad (11)$$

we see that the closed state depends on the occupancy in the following way

$$x_3 = -\frac{b_3 + \eta N}{2a_3} \quad (12)$$

and substituting this into Equation (10.b), we obtain the value of the conformational potential $U(x)$ at this point

$$U(x_3) = kT[-\kappa(N - N_0)^2 + c_3\varphi] + U_3^0, \quad (13.a)$$

where constants κ , N_0 and U_3^0 are determined by previous parameters a_3 , b_3 , μ and η that are voltage independent. N_0 determines the position of the extremum of $U(x_3)$ and consequently the extremum of the rate constant. Thus, this formula assumes that the maximum of $\alpha([K^+]_{\text{out}})$ is independent of the membrane potential that is observed experimentally.

At the same time, the energy $U(x)$ at the point x_1 is in the following form:

$$U(x_1) = kTc_1\varphi + U_1^0, \quad U_1^0 = a_1x_1^2 + b_1x_1. \quad (13.b)$$

Substituting expressions (13) into Equations (4) and introducing new variables we find the rate constants.

$$\alpha = \alpha_0 \cdot \exp[-\kappa(N - N_0)^2 + c_3\varphi],$$

$$\beta = \beta_0 \cdot \exp[c_1\varphi] \quad (14)$$

where α_0 and β_0 are new model parameters that include the initial energies U_i^0 . Note, that all parameters in Equation (14) are mutually independent sets and we can approximate α and β independently.

Thus the conformational potential determining the channel kinetics according to Equations (4) depends on the square of occupancy N . This dependence is based on the observed nonlinearity in Figures 2–4. To show this, we determine the dependence of the occupancy N on $[K^+]_{\text{out}}$. It is obvious that the concentration increase causes an increase of occupancy N until a saturation is achieved. This is due to the limited size of the binding site within the channel and, consequently, N cannot exceed a certain value. Thus, two main region typify the dependencies of the occupancy on concentration: a rising region and a flat region where saturation occurs. The latter reveals itself experimentally – the dependencies of the kinetic parameters on concentrations beyond 30 mM result in saturation (see Figures 4, 6). We found that the best approximation for the dependence is a known saturating function:

Figure 7. Relationships between concentration $[K^+]_{out}$ and the ion occupancy N (a) and the rate constant α (b), see text. Parameters are the same as for Figure 4.

$$N = \frac{[K^+]_{out}^\gamma}{\Omega + [K^+]_{out}^\gamma} \quad (15)$$

where γ , Ω are model parameters. The coefficient γ was found to be 6. The corresponding dependency of the occupancy N on $[K^+]_{out}$ is shown in Figure 7a. In Figure 7b one can see the corresponding concentrational dependency of the rate constant α (curves) described by formulas (14) together with the experimental data (circles).

4. Discussion

The Hodgkin and Huxley empirical theory of excitable membranes (H–H theory, [11]) is based upon kinetic equations connecting the current through the membrane with the applied electric potential. Coefficients appearing in the equations were found by fitting to the experimental data. Physical interpretation of the theory is based upon the notion of independent ‘gating particles’ that are responsible for the kinetics of current through the single ion channel. There are two types of particles (or gating processes): opening the channel in case of membrane depolarisation (activation) and closing it (inactivation). Thus the H–H model suggests, on the one hand, an adequate description of excitable membrane behaviour and, on the other hand, an illustrative idea of ion channel functioning. Nevertheless the H–H theory failed to give a microscopic interpretation. There are many of theoretical interpretations [3, 18, 19] as well as empirical models [1, 17, 20, 32]. (We cite here just a few.) In addition the H–H model (as well as cited models) does not describe the concentrational dependencies of the channel parameters. However, presently there are many of experiments demonstrating the influence of bulk concentrations

on channel functioning. Here are some examples. The strong nonlinearity of single channel CVR in human skeletal muscle was observed in [8]. It was shown that the output sodium current at +130 mV is a linear function of external sodium concentration although the electrochemical gradient should induce an opposite effect. The concentration dependence of bi-directional flux ration was observed [25]. Different ions have different interactions with the channel [26]. It was shown in [7] that the ion channel potential profile structure is changed for passing ion concentrations. The non-monotonous CVR of potassium channels are varied upon changing of sodium concentrations [22]. The shifts of dependencies of kinetic characteristics (life time, open state probability, etc.) and currents on the voltage to the reversal potential are typical displays of ion concentration influence on ion channel kinetics [10, 31]. In [27, 33] the following phenomena were observed: the channel life time rose while ion flux approaches zero. At the same time the closed state probability became practically zero. Thus, near zero current the channel has only one state – open.

One of the authors of this paper has also observed the influence of calcium concentration on the calcium current deactivation [15].

These facts as well as others show that ion concentrations can control channel functioning and correspondingly by a different approach should be developed. As mentioned above, P. Lauger was one of the first who took into account ion-channel interaction theoretically [16]. Such approaches were applied to different situations (see as examples [5–7]). But as a rule, modern theoretical concepts arrive at the postulates of existence of a set of *discrete constant* conductance states. Therefore ion-channel interaction can cause only changes of transition rates α and β but not the channel states themselves. In other words, ICI changes the energy levels $U(x_1)$, $U(x_2)$ and $U(x_3)$ but not the positions x_1 , x_2 and x_3 (see Figure 5). Moreover these approaches do not suggest any interpretation of the nature of discrete channel states. However, the self-consistent description of ICI [4, 14, 29, 30] can solve many problems mentioned above: the shift of $n_\infty(\varphi)$ under ion concentration variations, the disappearance of the closed state at zero current, the nature of channel conformational states, etc. Such an approach allows us to describe our experiments as well. We show that the non-monotonous dependencies of the transition rates from one state to the other on the external potassium concentrations are connected with the deformation of the effective conformational potential $U(x)$. A twofold manifestation of ICI causes this deformation: it does not only change the energy level $U(x_3)$, Equation (13.a), but the closed state x_3 itself, Equation (12).

From physical reasons we concluded that the influence of potassium concentrations on channel functioning caused by ICI is described by the relationships (14) between rate constants and ion occupancy. The typical dependence of N on ion concentration (for example, $[K^+]_{out}$) described by Equation (15) is shown in Figure 7a.

It is interesting that a strong non-linearity in channel kinetics is not represented in the channel CVR. But this phenomenon is explained by the fact that $[K^+]_{out}$

influences only the closed state which does not determine the value of single channel currents.

Thus, our results confirm the view that ICI can significantly influence channel functioning (ions regulate the channel) and reflect the structure-function relationships of these amazing molecular objects.

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Address for correspondence: V.N. Kharkyanen, National Academy of Science, Division of Physics of Biological Systems, Institute for Physics, Nauki Prospekt 46, Kiev 22, 252028, Ukraine
Fax: +380 44 29 35828; E-mail: antin@gluk.apc.org