Energetic Profile of the Open Calcium Channel. Experimental and Theoretical Studies

E. V. NARUŠEVIČIUS and M. Š. RAPOPORT

Institute of Cardiology, Eiveniu 4, 233007 Kaunas 7, Lithuanian SSR, USSR

Abstract. Dependence of inward ionic currents on the external and internal concentration of Ca or Sr ions and the membrane potential was examined in isolated dialysed neurons of *Helix pomatia*. The relation between the inward current and the external Ca or Sr ions concentration shows saturation and can be described by Langmuir's adsorbtion isotherm equation. In the examined range the dissociation constants (K_o^{Ca}) did not depend on membrane depolarization (V). The inward current carried either by Ca^{2+} or Sr^{2+} ions was blocked when the internal Ca ions concentration was increased. The block is independent on V and can be described by an equation of the Langmuir's type. The results are interpreted in terms of a three-barrier channel model which takes into the account changes in the membrane surface charge. The transit of an ion through the calcium channel seems to be associated with the binding of the ion to specific sites located both in the external and internal mouths, which have different affinities to the permeating ions.

Key words: Calcium channel — Channel models — *Helix pomatia*

Introduction

It is well established that the slow inward current through the calcium channels in different excitable membranes follows the Langmuir's function (for review see French et al. 1976). We failed, however, recently (Naruševičius and Zablockaite 1979) to explain the dependence of the inward current in snail neurons on the membrane potential and calcium by means of a two barrier channel model. Obviously it was due to the fact, that the inward and outward currents were not sufficiently separated.

Recently it became possible to record the inward calcium currents dissected from other ionic currents in dialysed snail neurons (Krishtal and Pidoplichko 1975). We used this method to reinvestigate the effects of divalent cations and membrane potential on the isolated calcium inward currents. The results obtained under these conditions are explained by means of a three barrier model describing the passage of divalent cations through the calcium channel.

Material and Methods

The experiments were performed on isolated neurons of *Helix pomatia*, which were dialysed and voltage-clamped as described elsewhere (Krishtal and Pidoplichko 1975). Isolation and quantification of the inward current component (Kostyuk et al. 1976) was described in detail by Naruševičius and Rapoport (1980). The internal Ca ions concentration was regulated by means of Ca-EGTA buffer.

Results

Fig. 1b shows a successive increase of the calcium inward currents amplitude with the increase of the external concentration of Ca ions, $[Ca]_o$. The effects of 2, 4 and 10 mmol/l $[Ca^{2+}]_o$ were examined. The potential-dependent characteristics of the

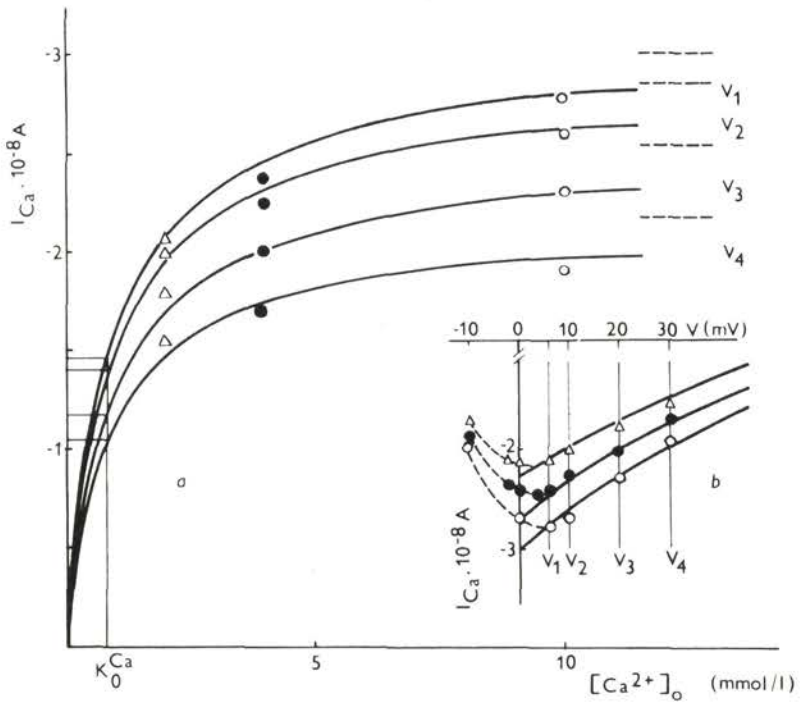

Fig. 1. Concentration (*a*) and voltage dependence (*b*) of the Ca-inward current. $[Ca^{2+}]_o = 2$ mmol/l (Δ), 4 mmol/l (●), 10 mmol/l (○). $V_1 - V_4$: testing depolarisation (V). The dotted lines represent the value of I_{max}^{Ca} at different V. The continuos lines are calculated from equation (1) for $K_o^{Ca} = 0.8.10^{-3}$ mol/l; $\alpha_2 = 0.18$; $I_{max}^{Ca} = 3.3 \times 10^{-8}$ A; at $V = 0$

inward current were shifted with the increase in $[Ca^{2+}]_o$ by 3.5, 3.0 and 2.0 mV respectively in the positive direction (mean values). The internal concentration of calcium ions, $[Ca^{2+}]_i$ was kept at the 5.10^{-10} mol/l level.

In order to determine the concentration dependence of the inward current, the amplitudes of inward currents corresponding to a given depolarization (V) were plotted against the current carrying ion concentration (Fig. 1a). The values of V were chosen so that they should be higher than those corresponding to the maximum of the volt-ampere characteristic for the highest current carrying ion concentration in the given experiments (values $V_1 - V_4$, Fig. 1b).

The experimental concentration dependences of Ca inward currents (I^{Ca}) could be described by the empirical equation:

$$I^{Ca} = I^{Ca}_{max} \frac{[Ca]_o}{K^{Ca}_o + [Ca]_o} \exp - (\alpha_2 V/12.5) \quad (1)$$

In accordance with equation (1) the curves given in the Fig. 1a show saturation at $I = I^{Ca}_{max.}$. With the increase in V the I^{Ca}_{max} decreases. The potential dependence of I^{Ca}_{max} follows the exponential curve. In the demonstrated case (Fig. 1b) the exponent (α_2) equals 0.18. The mean exponent value obtained in 16 cells was (0.20 ± 0.02). The corresponding constant (K^{Ca}_o) in the demonstrated case equals 0.8×10^{-3} mol/l, and it is independent of the membrane potential level. Similar results were obtained in the other 15 cases, the respective value K^{Ca}_o being independent on V. The mean value K^{Ca}_o equals $(1.3 \pm 0.5) \times 10^{-3}$ mol/l. Considering that the surface of the dialyzed somatic neuron equals approximately 7.7×10^{-5} cm^2, the mean current (I^{Ca}_{max}) amounts to $(3.3 \pm 1.0) \times 10^{-4}$ A/cm^2 at $V = 0$. The same analysis was performed after substitution of Ca ions with Sr ions in the external medium. The internal concentration of free $[Ca^{2+}]$ was kept at 5.0×10^{-10} mol/l. The effects of 5, 10, 15, 20 and 30 mmol/l $[Sr^{2+}]_o$ were examined with the following results. The corresponding shifts of the potential-dependent characteristics of the inward current in the direction of the positive values were 1.0; 1.0; 0.5 and 1.0 mV (mean values), respectively. Dependence of the strontium inward current on the external concentration of Sr ions obeys the same equation (1), as does the Ca inward current. In this case, however, the K^{Sr}_o value depends on the membrane potential:

$$K^{Sr}_o = K_o \exp(\alpha_1 V/12{,}5), \quad (2)$$

where K_o is the value of K^{Sr}_o at $V = 0$. With the increase in V the value K^{Sr}_o increases exponentially with the power (α_1), which equals (0.22 ± 0.4), and the value I^{Sr}_{max} decreases exponentially with the power $\alpha_2 = (0.21 \pm 0.04)$. Both, α_1 and α_2 were calculated as mean values from 27 experiments. For the same number of neurons $K_o = (6.2 \pm 1.4)\ 10^{-3}$ mol/l, and I^{Sr}_{max} at $V = 0$ equals $(4.80 \pm 0.7)\ 10^{-4}$ A/cm^2.

The introduction into the cell of 10 mmol/l EGTA resulted in no changes in the Ca inward current. The same holds true, when the $[Ca^{2+}]_i$ was increased to

5.0×10^{-10} mol/l by addition of Ca-EGTA buffer into the dialyzing solution. Further increase in $[Ca^{2+}]_i$ blocked the inward current. In these experiments, the increase in $[Ca^{2+}]_i$ did not result in a significant displacement of the inward current-voltage curves. Shift of the current-voltage curves to less positive values was observed on occasional instances and it had an irregular character, as the inward current amplitude diminished sharply with the increase in $[Ca^{2+}]_i$.

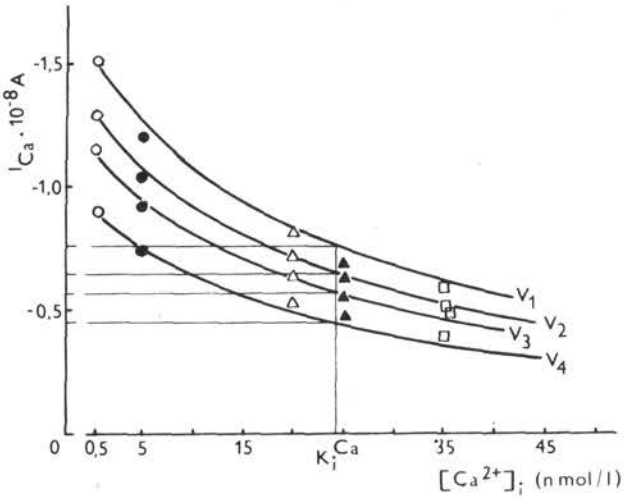

Fig. 2. Dependence of the Ca inward current blockade on the intracellular concentration of the Ca^{2+} ions. $[Ca^{2+}]_i = 3.5 \times 10^{-8}$ mol/l (□); 2.5×10^{-8} mol/l (▲); 2.0×10^{-8} mol/l (△); 5.0×10^{-9} mol/l (●); 5.0×10^{-10} mol/l (○). The continuos lines were drawn according to equation (3) at $K_o^{Ca} = 0.9 \times 10^{-3}$ mol/l; $K_i^{Ca} = 2.4 \times 10^{-8}$ mol/l; $I_{max}^{Ca} = 2.0 \times 10^{-8}$ A at $V = 0$, $[Ca^{2+}]_o = 5.0 \times 10^{-3}$ mol/l. $V_1 - V_4$: testing depolarizations.

Fig. 2 shows depresion of the Ca inward current in one neuron during its dialysis with Tris-Cl solution containing Ca-EGTA buffer keeping up the free calcium level at 5.0×10^{-10}, 5.0×10^{-9}, 2.0×10^{-8}, 2.5×10^{-8} and 3.5×10^{-8} mol/l, respectively. The continous lines in the figure are drawn according to the equation:

$$I^{Ca} = I' \frac{K_i^{Ca}}{K_i^{Ca} + [Ca]_i} \exp -(\alpha_2 V/12{,}5) \tag{3}$$

where $I' = I_{max}^{Ca} [Ca]_o / (K_o^{Ca} + [Ca]_o)$ represents the current at $[Ca]_i \to 0$. In the demonstrated case (Fig. 2), the constant ($\bar{K}_i^{Ca}$) equals 2.4×10^{-8} mol/l. The mean value of the constant ($\bar{K}_i^{Ca}$) from 7 cells equals $(2.8 \pm 0.4) 10^{-8}$ mol/l. In all cases examined the value K_i^{Ca} did not depend on the magnitude of V. Increase in $[Ca^{2+}]_i$ also causes the blockade of the Sr inward current, passing through the calcium channel. The blockade of the Sr current follows equation (3). The mean value of

the constant ($\bar{K}_i$) calculated for 5 neurons, equals $(4.3 \pm 0.9) \times 10^{-8}$ mol/l; K_i constants were independent of the membrane potential changes.

Discussion and Theory

The interpretation of data is based on the three-barrier model of the ionic channel (Markin and Chizmadzhev 1974). According to our model (Naruševičius and Rapoport 1979), the ion moving through the calcium channel interacts in succesion with the membrane surface charge, and with the binding sites at the external and intracellular channel mouths. Generally, the ion or its substitute has to get over three potential barriers. The energetic profile of an ion in such a channel can be presented in the form of a curve drawn in Fig. 3, where the regions I and III correspond to the external and intracellular channel mouth respectively and the region II to the central barrier. If we neglect the surface charge changes, and if the heights of potential barriers and $[Ca]_o$, $[Ca]_i$ are such that the first well is practically filled only from external and the other one only from internal side, such filling is not correlative and $\nu_o < K_1$.

In this case, the ionic current transferred through the calcium channel in the examined model follows the equation:

$$I^x = 2qN\left[\frac{[X]_o}{K_o^x + [X]_o}\frac{K_i^x}{K_i^x + [X]_i}\nu_o \exp - (2q\alpha_2 V/kT) - \right.$$
$$\left. - \frac{[X]_i}{K_i^x + [X]_i}\frac{K_o^x}{K_o^x + [X]_o}\nu_i \exp(2q\alpha_3 V/kT)\right] \quad (4)$$

where $K_o^x = (K_2 + \nu_o)/K_1$ (5)

Here, q is the elementary charge;
N is the number of open channels;
$[X]_o$ and $[X]_i$ are intra and extracellular concentrations of the current carrying ion;
K_o^x is dissociation constant of the ion complex X^{2+} with the binding site at the external channel mouth;
K_i^x is dissociation constant of the ion complex X^{2+} with the binding site at the intracellular channel mouth;
ν_o and ν_i are rate constants of the jumps over the central barriers
K_1 and K_2 are rate constants of the jumps over the first barrier;
α_2 and α_3 are the components of the membrane potential drop on the central barriers;
k is Bolzman's constant;
T is absolute temperature.

At the constant $[X]_i$ the equation (4) is reduced to (1), where I^{Ca}_{max} is proportional to v_o (the outward current in the range of the examined depolarization is several orders of magnitude smaller than the inward one). In accordance with (4) the transfer of ions through the channel is associated with their adsorbtion by channel structures. Increase in concentration of the permeating ion decreases the number of free binding sites at the external channel mouth, due to adsorbtion, resulting in saturation of the inward current. I^{Ca}_{max} in (1) represents thus the value of the inward current when all binding sites have already been occupied by the current carrying ions. The saturation effect of the Ca inward current was first observed in muscle fibres of barnacle (Hagiwara et al. 1974; Hagiwara and Takahashi 1976) and subsequently revealed in starfish eggs (Hagiwara et al. 1975), in eggs of some tunicate species (Okamato et al. 1976), as well as in the heart muscle fibres (Carmeliet et al. 1978). Ca inward current saturation is observed in the soma membrane of the dialyzed neurons of *Helix aspersa* when the $[Ca^{2+}]_o$ is increased to 30—40 mmol/l. K_o^{Ca} at $V=0$ equals 5.4 mmol/l (Akaike et al. 1978). Saturation of the Sr inward current was observed in the Purkinje fibres of the bull; K_o^{Sr} at $V=0$ was 7.0 mmol/l (Vereecke and Carmeliet 1971 a, b). In the present experiments it was demonstrated, that $\bar{I}^{Ca}_{max} : \bar{I}^{Sr}_{max} = 1 : 1.43$. Higher inward current carried by Sr^{2+} ions as compared with that carried by Ca^{2+} ions was observed in the guinea pig atrium fibres (Pappano 1970), and in the neuron membrane of molluscs (Akaike et al. 1978). In the muscle fibres of barnacle the ratio of maximum currents $I^{Ca}_{max} : I^{Sr}_{max} = 1.0 : 1.05$ (Hagiwara et al. 1974). In the dialyzed neurons of *L. stagnalis* the maximum currents ratio $I^{Ca}_{max} : I^{Sr}_{max} = 1 : (2 \pm 0.3)$ (Naruševičius et al. 1979). According to the model, the currents ratio may be explained by a decrease in the height of the central barrier for the Sr^{2+} ions ($h_2' < h_2$) (Fig. 3). In this way, the central potential barrier could play the role of a selective filter in the calcium channel. Studying the calcium channel of the rat synapse, Nachen and Blaustein (1979) came to similar conclusion concerning the presence of a high potential barrier inside the channel determining its selectivity.

The potential dependence of the maximum current in the equation (4) is determined by the factor $v_o \exp(-2q\alpha_2 V/kT)$, which determines the exponential form of the descending part of the inward current-voltage relation. A similar form of the current-voltage characteristic was obtained in the dialyzed neurons of *Helix aspersa* and *L. stagnalis* (Hagiwara and Naka 1964; Naruševičius et al. 1979).

The blockade of the inward current by intracellular calcium is expressed by the multiplicator $K_i^x/(K_i^x + [X]_i)$ in equation (4). As the $\bar{K}_i^{Ca}$ value is in the 10^{-8} mol/l range, the second well is already filled at a low $[Ca^{2+}]_i$ and as a result, the passage of the current carrying ion from the first well through the central barrier is blocked. This would explain the blocking effect of small intracellular concentrations of Ca^{2+} ions on the calcium channel (Hagiwara and Naka 1964; Kostyuk and Krishtal 1977). The model is also supported by the fact that increase in $[Ca^{2+}]_i$ results in the

blockade of the inward current carried both by Ca^{2+} or Sr^{2+} ions (the differences between K_i^{Ca} and K_i' are not statistically significant). Hagiwara and Nakajima (1966) did not observe, however, in muscle fibres of barnacle a depression of the strontium spikes even at 10 times higher $[Ca^{2+}]_i$ than that which blocked fully the calcium spikes.

As the K_i^{Ca} and K_i' were independent of change in V, it may be concluded that the binding sites at the intracellular channel mouth might lie, outside the sphere of influence of the membrane potential.

The experiments have shown that the increase in the external concentration of Ca^{2+} or Sr^{2+} at a constant $[Ca^{2+}]_i$ shifts the current-voltage characteristics towards positive values of V. This effect can be explained by assuming the existence of a surface charge on the examined membrane in the calcium channel region. Guy-Chapman's diffuse layer theory was used to describe the electrostatic interaction of the current carrying cations with the negatively charged groups fixed on the membrane surface. According to this theory, the value of the membrane surface potential (ψ) is related to the density of fixed charges (σ) and the cation concentration in the solution by the following equation:

$$\sigma = \frac{1}{272}\left[C_+ (e^{F\psi/RT} + e^{-F\psi/RT} - 2) + C_{++} (2e^{F\psi/RT} + e^{-2F\psi/RT} - 3)\right]^{1/2} \quad (6)$$

where C_+ is concentration of univalent cations (kept constant),
C_{++} is concentration of divalent cations in the solution,
F is Faraday constant,
R is gas constant.

With regard to the specific binding of cations with fixed charges, the expression for σ must be changed according to the Michaelis-Menten equation type to:

$$\sigma = \frac{\sigma_T}{1 + C_{++} \exp - (2F\psi/RT) K_d^{\psi}} \quad (7)$$

where σ_T is the highest density of charges observed in the absence of binding cations;
K_d^{ψ} is the dissociation constant for the neutralisation reaction.

Solving the equation (6) for Sr inward current shows that a satifactory description of the displacement of current-voltage characteristics along the potential axis by changing $[Sr^{2+}]_o$ is attained when $\sigma = 1\bar{e}/12.25\ nm^2$ (Fig. 4). Using this, value of σ, the solution of equation (1) for Ca inward current gives significant deviation of the calculated changes of ψ from the experimentally obtained displacements of current-voltage characteristics. Due to this, the equation (7) was used; it takes into account both the shielding and the binding of the current carrying cations with fixed charges. This way, we succeeded in obtaining a satisfactory coincidence of theoretically calculated changes in ψ with experimentally obtained shift of the Ca

current-voltage relation with changes in $[Ca^{2+}]_o$. At the same density of fixed charges ($1\bar{e}/12.25\ nm^2$), the dissociation constant for Ca^{2+} ions (K_d^{ψ}) was equal to 4.9×10^{-2} mol/l (Fig. 4).

Displacement of current-voltage characteristics of Sr^{2+} ions can be thus explained by electrostatic shielding of fixed charges, and of Ca^{2+} ions, in addition to shielding, by specific binding with fixed charges on the membrane surface. A similar interaction with the surface charges in the region of calcium channels was found in the egg membrane of tunicates (Ohmori and Yoshii 1977) and in the neuron membrane of molluscs (Kostyuk et al. 1980). Due to interactions of fixed negative charges with current carrying cations, the presence of the former results in an increase in the concentration of the given cations in the region of the external mouth of the calcium channel which differs from their concentration in the external solution surrounding the cell. The value of this concentration ($[X]_o'$) is determined by the expression:

$$[X]_o' = [X]_o \exp - (2F\psi/RT) \tag{8}$$

On the other hand, the field created by the surface charge is distributed on the first and second barriers and changes their heights, thus influencing the channel permeability. The height change of the potential barriers under the influence of ψ can be expressed as follows:

$$K_2 = K_2' \exp - (\alpha_1 2F\psi/RT) \tag{9}$$

$$v_o = v_o' \exp(\alpha_2 2F\psi/RT) \tag{10}$$

By substituting the equations (8—10) for (5), we obtain the expression for the dissociation constant ($K_o^{x(\psi)}$), taking into account the membrane surface charge:

$$K_o^{x(\psi)} = \frac{K_2' \exp[(2-\alpha_1)F\psi/RT] + v_o' \exp[(2+\alpha_2)F\psi/RT]}{K_1} \tag{11}$$

Since in equation (11) α_1 and $\alpha_2 \ll 2$, the basic influence of the surface charge is evidently expressed by $[X]_o^m$; consequently in the first approximation, it is possible to neglect the changes in the channel permeability influenced by ψ. In order to show the influence of $[X]_o'$, the values of I^x, calculated from the equation 1 and 2 for the mean values of K_o^x; α_1 and α_2, were plotted on the graph in relation to $[X]_o'$. Analysis of the curves showed that the potential dependence of I_{max} and I_{max}^{Sr}, as well as the potential dependence of the dissociation constants for the intracellular Ca^{2+} or Sr^{2+} ions remain unchanged (changed correction for the influence of $[X]_o'$). As shown by the results, in the range of the examined depolarisations the K_o^{Ca} was in fact independent of the changes in V, while the K_o^{Sr} changed according to equation (2). Proceeding from the developed model, the potential dependence of K_o^x can be written as

$$K_o^x = [K_2 \exp(2q\alpha_1 V/kT) + v_o \exp - (2q\alpha_2 V/kT)] : K_1 \tag{12}$$

where α_1 is the portion of the membrane potential drop on the first barrier (Fig. 3).

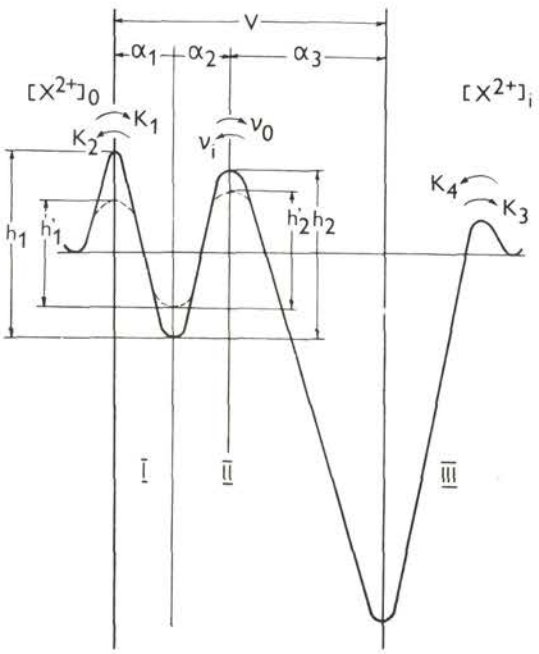

Fig. 3. The energetic profile of the X^{2+} ion in the calcium channel. On the left—the external solution, on the right—the intracellular one. The regions I and III correspond to the external and internal channel mouth respectively and region II corresponds to the central barrier. $\alpha_1 - \alpha_3$ — the portions of the membrane potential drop on the potential barriers. The continuous line represents the energetic profile of Ca^{2+}, dotted lines show the change in energetic profile of Sr^{2+}.
$h_1 - h_2$ — heights of the potential barriers for Ca^{2+}
$h_1' - h_2'$ — heights of the potential barriers for Sr^{2+}.

The demonstrated dependence of dissociation constants on the membrane potential can be reproduced on the assumption that the first barrier for Ca^{2+} is higher than the central barrier for Sr^{2+} ($h_1 > h_2'$). If Sr^{2+} ions pass through the channel, the height of the first barrier appears for them to be lower than for the Ca^{2+} ions ($h_1 > h_1'$). As a result of these changes the following relations of barrier heights for the permeating ions arise for Ca^{2+} ($h_1 > h_1'$) (Fig. 3), for Sr^{2+} ($h_1' < h_2'$). From equations (9, 10, 12) the following expression for the potential dependence of $K_o^{x(\psi)}$ can be obtained:

$$K_o^{x(\psi)} = \frac{K_2' \exp[2q\alpha_1(V-\psi)/kT] + \nu_o' \exp[2q\alpha_2(\psi - V)/kT]}{K_1} \qquad (13)$$

Substituting in (13) the values of K_1, K_2' and ν_o' and also the values of ψ

corresponding to the different $[Ca^{2+}]_o$ and $[Sr^{2+}]_o$ (Fig. 4), the influence of surface charge on the calcium channel permeability was calculated. The values of the

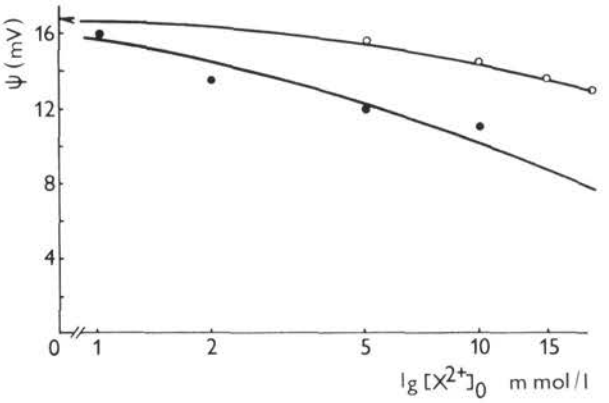

Fig. 4. Dependence of the surface potential on the concentration of the current carrying ions in the external solution (log scale). The points represent the experimentally obtained shifts of the current-voltage curves for the inward current carried by Sr^{2+} (○) and Ca^{2+} (●) ions. The continous lines are drawn according to equations (6,7) at $\sigma = 1\bar{e}/12{,}25\ nm^2$; K_d^{ψ} equals 4.9×10^{-2} mol/l for Ca^{2+} ions.

kinetic constants K_1', K_2' and v_o, were selected from the dissociation constants after correction for the influence of $[X]_o'$ and with regard to their dependence on the membrane potential. Analysis of equation (13) showed the apparent value of the dissociation constant for the extracellular calcium $K_o^{Ca(\psi)}$ to be 4.8×10^{-3} mol/l and that for the extracellular strontium $K_o^{Sr(\psi)}$ 29.2×10^{-3} mol/l.

The plot of the dependence of $K_o^{Sr(\psi)}$ and of $K_o^{Ca(\psi)}$ on the membrane potential drawn according to equation (13) is presented in Fig. 5. Corresponding with the described energetic profile, the quantity $K_o^{Ca(\psi)}$ is independent of the membrane potential at small values of V. At high values of V, the field dependence $K_o^{Ca(\psi)}$ is determined by the first term in equation (13), which is expressed in the exponential increase in the values $K_o^{Ca(\psi)}$ with the exponent α_1. The latter agrees well with the data obtained in the dialyzed neurons of *Helix aspersa* (Akaike et al. 1978), where a significant increase in the potential dependence of the dissociation constant in the depolarisation range $+50 - +100$ mV was shown. For $K_o^{Sr(\psi)}$ the demonstrated energetic profile indicates an exponential character of the potential dependence of the dissociation constant for all depolarizations (V).

The values of the dissociation constant were used to calculate the changes in the free energy (ΔG), which is necessary for the formation of bonds of current carrying Sr^{2+} and Ca^{2+} ions with the binding site at the external channel mouth. For $K_o^{Sr(\psi)}$ and $K_o^{Ca(\psi)}$, ΔG equals 2.1 and 3.1 kcal/mol respectively, which is typical for electrostatic interactions. ΔG for bonds of the Ca^{2+} ions with fixed charges on the

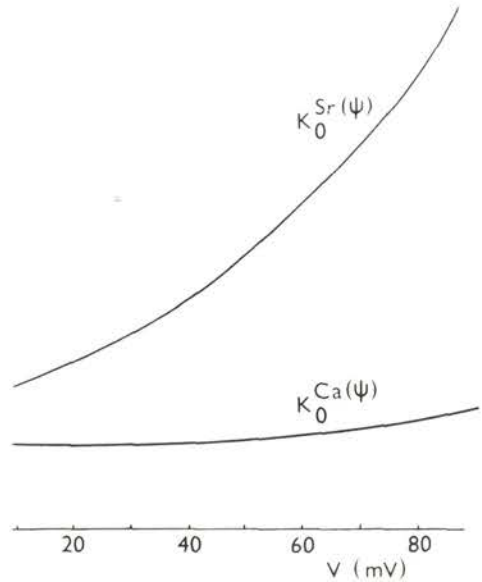

Fig. 5. Potential dependence of dissociation constants for the current carrying Ca^{2+} and Sr^{2+} ions. The continuos lines are drawn according to equation (13) for $\alpha_1 = 0.22$ and $\alpha_2 = 0.20$. The values of $K_o^{Ca(\psi)}$ were obtained for $K_1 = 0.40$; $K_2' = 0.29$; $v_o' = 1.00$. The values $K_o^{Sr(\psi)}$ were obtained for $K_1 = 10.00$; $K_2' = 0.39$; $v_o' = 1.43$. The value ψ was calculated from the curve shown in Fig. 4.

membrane surface equals 1.8 kcal/mol. This is approximately 1.8 times smaller than ΔG, which is typical for the bond of the Ca^{2+} ions with the absorption site at the external channel mouth. The latter, obviously, supports the view, that the observed effects of the Ca^{2+} ions, cannot be explained only by the changes in the density of the fixed charges. To describe the ionic inward current, it is therefore necessary to take into account the specific interaction of the permeating ions with the calcium channel.

The analysis has thus shown, that the ion transfer through the open calcium channel, according to the examined model, appears as a series of consecutive steps. First step is represented by interaction of the permeating ions with the negative charges on the membranes surface. The mechanism of this interaction for the permeating Sr^{2+} ions consists in the electrostatic shielding of the fixed charges, and for the Ca^{2+} ions along with the shielding — in the formation of a bond with fixed charges. At the second stage, the permeating ions are adsorbed on the binding site at the external channel mouth. The finite number of these sites at sufficiently great values $[X]_o$ determines the effect of the inward current saturation. At the third stage, the permeating ions jump through the central barrier, which plays the role of a selective filter in the channel; they are then adsorbed at the internal binding site,

if it is vacant. Adsorbtion of the permeating ions on the binding site at the intracellular mouth results in the blockade of the ionic channel. At the fourth stage, the ions are obviously released, into the cell interior.

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