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# Energy Profile of the Calcium Channel in the Membrane of Mollusc Neurons

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**Summary.** The current-voltage characteristics of the inward calcium and barium currents at different concentrations of these ions in the extracellular solution have been measured on isolated neurons of the snail *Helix pomatia* intracellularly perfused with potassium-free solution containing 10 mM EDTA. On the basis of these characteristics the energy profile of the calcium channel has been calculated using a model based on the absolute reaction rate theory developed by Eyring. The effect of changes of the near-membrane concentration of the penetrating ions due to the existence of fixed charges on the outer side of the membrane has been taken into account. A satisfactory description of the concentration- and potential-dependence of the calcium inward currents has been obtained based on a three-barrier model for the energy profile of the calcium channel. Calculated dissociation constants for the complexes of $Ca^{2+}$ ions with the binding sites of the calcium channel have the following values: $K_{out}=10$ mM and $K_{in}=2.5$ mM; and for the complexes of $Ba^{2+}$ ions, $K_{out}=91$ mM and $K_{in}=1.5$ mM. The outer binding site corresponds to the acidic group with $pK=5.8$. Comparison between these data and the values of pK for divalent cation complexes with different anionic groups of amino acids allowed us to suggest that the outer binding site contains only one carboxylic group. It was shown that the strength of cation binding to this group determines the conductance of the calcium channel.

**Key Words** energy profile · calcium channel · mollusc neurons

## Introduction

The description of ionic membrane transport on the basis of absolute reaction rates theory was developed about 30 years ago by Eyring, Lumry and Woodbury (1949), but the application of this method to excitable biological membrane systems has been started only recently. Markin and Chizmadjev (1974), Hille (1975), Hille and Schwartz (1978) have used it for the determination of the energy profiles in the sodium and potassium channels in the membrane of nerve fibers.

The theoretical description of ionic transport phenomena in calcium channels of nerve cell soma membrane has been much less detailed, though there were some works concerning this subject (Naruševičius & Rapoport, 1979; Yasui, Brown, Akaike & Lee, 1979). In the present work we give a quantitative treatment of the ionic transport phenomena in calcium channel made on the basis of a three-barrier model, which takes into account the effects of surface charges of the membrane, the replacement of $Ca^{2+}$ ions in the extracellular medium by other divalent cations, and the introduction of $Ca^{2+}$ ions inside the cell on the value of the steady-state current passing through the calcium channel.

The present study allowed us to demonstrate not only the usefulness of Eyring's approach for the description of calcium channel current-voltage characteristics obtained for different carrier ions and blockers, but also to make certain conclusions about the nature of those functional groups of the calcium channel, which determine its conductivity. A preliminary communication of the work has been published elsewhere (Kostyuk, Mironov & Doroshenko, 1980).

## Theory

For the calculation of the energy profile of the calcium channel we adopted the single-file one-ion pore model similar to those developed by Markin and Chizmadjev (1974) and Hille (1975) for sodium channel.

We described the processes of ionic transport in the calcium channel on the basis of the three-barrier model. This model is the simplest one, and can be used for the calcium channel

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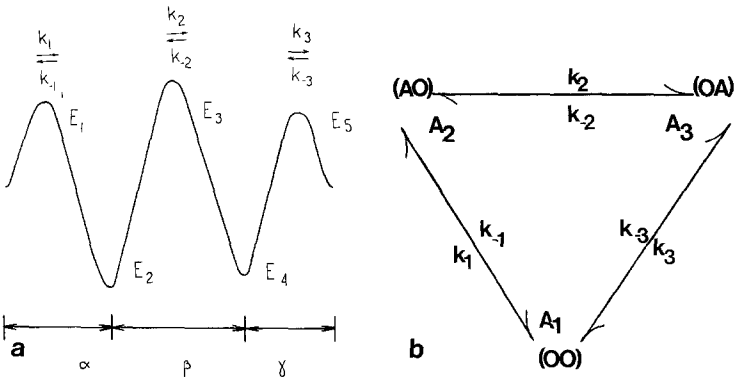


**Fig. 1.** (*a*) Three-barrier model for the energy profile of the calcium channel ($k_i$ are the rate constants for translocation of an ion through the corresponding potential barrier; $E_i$ are the energies of potential barriers and wells; $\alpha$, $\beta$, $\gamma$ are the fractions of the transmembrane potential drop on the corresponding barrier. (*b*) The diagram of ion-ionic channel states for three-barrier model of calcium channel. Index $A$ corresponds to the state when the well is occupied by ion; 0, when it is empty

because it is known to contain two Ca-binding sites of different affinity (Kostyuk et al., 1980).

According to Eyring et al. (1949), the steady-state value of the current through the single-file ionic channel is determined by the relations between the constants $k_i$ (Fig. 1*a*) describing the transition of the ion over the potential energy barriers. The values of these constants in the presence of external electric field in the membrane are determined in the following way:

$$
\begin{aligned}
k_1 &= \frac{kT}{h}\,\kappa_1\, C_{\text{out}} \exp(-E_1 - z\alpha V/2)\\
k_{-1} &= \frac{kT}{h}\,\kappa_{-1} \exp(-E_1 + E_2 + z\alpha V/2)\\
k_2 &= \frac{kT}{h}\,\kappa_2 \exp(-E_3 + E_2 - z\beta V/2)\\
k_{-2} &= \frac{kT}{h}\,\kappa_{-2} \exp(-E_3 + E_4 + z\beta V/2)\\
k_3 &= \frac{kT}{h}\,\kappa_3 \exp(-E_5 + E_4 - z\gamma V/2)\\
k_{-3} &= \frac{kT}{h}\,\kappa_{-3}\, C_{\text{in}} \exp(-E_5 + z\gamma V/2)
\end{aligned}
\qquad (1)
$$

where $E_i$ corresponds to the minima (even indices) and maxima (odd indices) of the ion energy profile in the channel (all energies correspond to free energies expressed in $RT$ units; $RT = 0.55$ kcal/mole at room temperatures); $\kappa_i$ are the transmission coefficients which depend on the steric factors of ion interaction with the functional groups of the channel; $\alpha$, $\beta$, $\gamma$ are the fractions of membrane potential drops between the corresponding energy wells (Fig. 1*a*); $C_{\text{out}}$ ($C_{\text{in}}$) are the local concentrations of the carrier ions in the immediate vicinity of the outer (inner) mouth of the channel; $V$ is the potential in $RT/F$ units ($RT/F = 25$ mV at room temperatures); $k$ and $h$ are the Boltzmann and Planck's constants, respectively.

The use of the values of ionic concentrations instead of activities in Eq. (1) is a correct approximation for diluted solutions ($C < 0.2$ M). Thus assuming the surrounding ionic solutions are ideal we can also put the value of the free energy (i.e. the chemical potential) of carrier ions in them equal to zero.

It can be seen from Eq. (1) that the current through the ionic channel in the case of a three-barrier model depends implicitly upon 14 parameters. If we accept the suggestions usually made about their values (Markin & Chizmadjev, 1974; Hille, 1975), i.e. the linear drop of transmembrane potential ($\alpha + \beta + \gamma = 1$), the equality of all transmission coefficients ($\kappa_i = \kappa$) then a simplified model with 7 parameters can be constructed.

The expression which gives the value of the steady-state current through the ionic channel can be readily obtained using the diagram technique (*see*, for instance, the appendix in the book of Markin and Chizmadjev, 1974). Using this technique, first the possible states of the ion-ionic channel system are constructed and then the corresponding occupation numbers, and the resulting ion flux are calculated. This diagram for the three-barrier single-particle model is shown in Fig. 1*b*. The occupation numbers $A_i$ for this model can be expressed as functions of the transition rate constants in the following way:

$$
\begin{aligned}
A_1 &= k_{-1}k_{-2} + k_2 k_3 + k_{-1}k_3\\
A_2 &= k_1 k_3 + k_{-2}k_{-3} + k_1 k_{-2}\\
A_3 &= k_1 k_2 + k_{-1}k_{-3} + k_2 k_{-3}.
\end{aligned}
\qquad (2)
$$

So far as the sum of all occupation numbers must be equal to unity, all $A_i$ values have to be divided by their sum.

According to the continuity principle, the value of the steady-state flux of the ions $j$ is equal to the difference between the inward and outward fluxes through each of the potential barriers. Their values are determined as the product of the occupation number of the corresponding energy well and the rate constant of the transition of the ion through the corresponding potential barrier. Thus,

$$j=(k_1 A_1-k_{-1} A_2)=(k_2 A_2-k_{-1} A_3)$$
$$=(k_3 A_3-k_{-3} A_1). \quad (3)$$

From Eqs. (2) and (3) it follows that the value of the ionic current through a single channel is equal to:

$$i=ze\frac{k_1 k_2 k_3-k_{-1} k_{-2} k_{-3}}{k_{-1} k_{-2}+k_2 k_3+k_{-1} k_3+k_1 k_3+k_{-2} k_{-3}+k_1 k_{-2}+k_1 k_2+k_{-1} k_3+k_2 k_{-1}}. \quad (4)$$

The expression for the value of the ionic current through a single channel in the presence of two or more carrier ions in the extracellular solution can be obtained in a similar way (*cf.* Hille, 1975), but the final expression is rather cumbersome and is omitted here.

## Materials and Methods

The experiments were made on isolated intracellularly perfused neurons from the snail *Helix pomatia*. The technique of cell isolation and perfusion, the composition of extra- and intracellular solutions and current recording system has been described in the preceding papers (Kostyuk & Krishtal, 1977*a*; Kostyuk, Krishtal & Pidoplichko, 1981; Kostyuk, Mironov, Doroshenko & Ponomarev, 1982).

As it follows from Eq. (4), the steady-state value of the ionic current through the open channel depends upon the local concentrations $C_{out}$ and $C_{in}$ of the carrier ions in the vicinity of the first and last potential barriers. The surface of the somatic membrane has fixed charges on it, and therefore the local ionic concentration near the membrane surface (and the ionic channel) will be different from that in the bulk solution; the experimentally measured potential difference between the extra- and intracellular solutions also includes the potential drop produced by fixed surface charges (Kostyuk et al., 1982).

Since, for the calculation of the energy profile of the calcium channel in the present work, the data obtained at $C_{in}=0$ were used, the effects of surface charges at the inner side of the membrane were neglected. The data about surface charges at the outer surface of the membrane have been taken from the previous work (Kostyuk et al., 1982) and were used for the determination of true position of current-voltage curves on the potential axis as well as of the values of local concentrations of ions $C^s_{out}$ near the external mouth of the calcium channel. The $C^s_{out}$ values were calculated using the equation:

**Table 1.** The values of the bulk ($C_{out}$) and near-membrane ($C^s_{out}$) extracellular concentrations of divalent ions (mM) and the corresponding values of surface potential ($\varphi$) (in mV) taken from Kostyuk, Mironov, Doroshenko & Ponomarev, 1982

| Ion | $C_{out}$ | 5 | 10 | 30 | 60 |
|---|---|---|---|---|---|
| $Ca^{2+}$ | $C^s_{out}$ | – | 28 | 53 | 90 |
| | $\varphi$ | – | −13 | −7 | −5 |
| $Ba^{2+}$ | $C^s_{out}$ | 31 | 58 | 117 | 170 |
| | $\varphi$ | −23 | −22 | −17 | −13 |

$$C^s_{out}=C_{out}\exp(-z\varphi F/RT) \quad (5)$$

and they are presented in Table 1.

As can be seen from the Table, the increase in ion concentration near the membrane is much more pronounced for $Ba^{2+}$ ions than for $Ca^{2+}$ ions. This is due to a more effective binding of $Ca^{2+}$ ions to the charged groups at the surface, leading to a larger decrease of the surface potential in the absolute magnitude.

The value of the steady-state ionic current can be written in the following general form:

$$I=Pm^a_\infty(V)\, i(C_{out}, C_{in}, V) \quad (6)$$

where $P$ is the proportionality coefficient, $m^a_\infty(V)$ is the relative number of open channels, $i(C_{out}, C_{in}, V)$ is the current through a single open channel. Proceeding from the data of Kostyuk and Krishtal (1977*a*), the value of $a=2$ was accepted for calcium channels. The potential-dependence of $m_\infty$ variable has been described by the expression:

$$m_\infty=[1+\exp(-b(V-V_{\frac{1}{2}}))]^{-1}, \quad (7)$$

where the steepness parameter $b=zF/RT=0.12$ mV$^{-1}$ for $z=3$, as has been found from the measurements of the kinetics of calcium ionic and gating currents in the same object (Kostyuk et al., 1981); $V_{\frac{1}{2}}$ is the testing potential producing the activation of a half of all calcium channels.

The parameters of the energy profile model were found by minimization of the mean square deviation of the theoretical current-voltage curve, calculated according to Eq. (6), from the experimental curve. For these calculations data obtained at $C_{out}=10$ mM for $Ca^{2+}$ and $Ba^{2+}$ ions have been used. Current carried by $Ca^{2+}$ or $Ba^{2+}$ ions through a single channel has been calculated according to Eq. (4). The obtained parameters of the model have been further used for the calculation of current-voltage curves for other extracellular concentrations of $Ca^{2+}$ and $Ba^{2+}$ ions; the single-channel current values calculated on the basis of our model for extracellular concentration of $Ba^{2+}$ and $Ca^{2+}$ ions equal to 130 mM coincided with those obtained experimentally by Kostyuk et al. (1978).

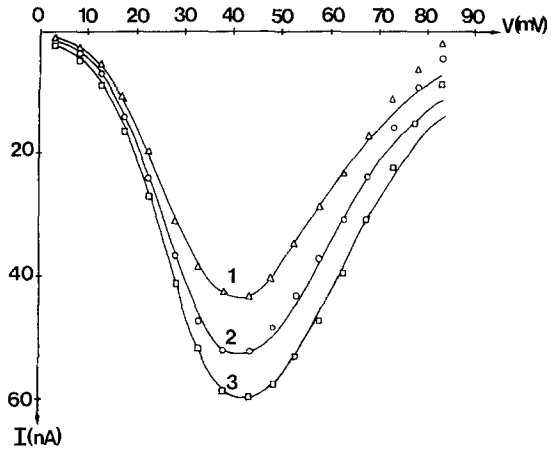


**Fig. 2.** Theoretical (curves) and experimental (points) current-voltage characteristics for calcium current. $C_{out}(Ca^{2+})=60$ mM (3), 30 mM (2), 10 mM (1), $C_{in}=0$

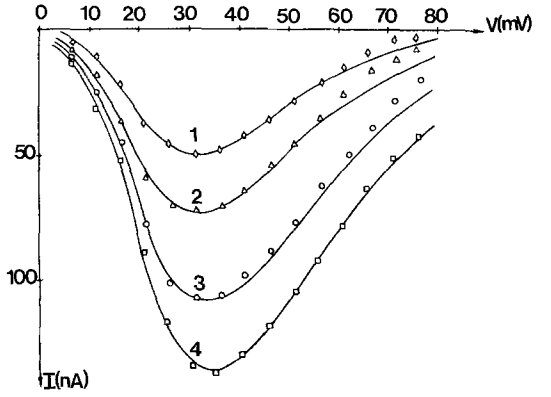


**Fig. 3.** Theoretical (curves) and experimental (points) current-voltage characteristics for barium current. $C_{out}(Ba^{2+})=60$ mM (4), 30 mM (3), 10 mM (2), 5 mM (1), $C_{in}=0$

In a similar way the parameters of the model for $H^+$ ions have been determined. The basic calculations have been made from current-voltage curves measured at $C_{out}(Ba^{2+})=30$ mM at pH of the extracellular solution equal to 5.6.

Despite the fact that our model contains a quite large number of parameters (seven for an individual ion), all of them can be determined from the single corresponding current-voltage characteristic and the value of single-channel current.

## Results

Figures 2 and 3 present the experimental data about the concentration dependence of calcium and barium inward current-voltage characteristics and the results of their approximation by the three-barrier model of the energy profile of the calcium channel. It should be noted that each current-voltage characteristic shown in Figs. 2, 3 and 5 was shifted towards more positive potential values by a magnitude of surface potential $|\varphi|$, which was calculated in the previous work (Kostyuk et al., 1982; *see also* Table 1). The current-voltage curves calculated at pH = 7 fit well to the experimental data in the potential range from −10 to +60 mV. In the case of testing potential more positive than +60 mV the approximation was less successful. This is due to the development of a nonspecific outward current carried by Trisions current in this region of potentials (Doroshenko, Kostyuk & Tsyndrenko, 1978). The energy profile of the calcium channel calculated on the basis of these data for $Ca^{2+}$ and $Ba^{2+}$ ions is given in Fig. 4. The standard deviation of the parameters of the model from their mean values obtained using the experimental data measured on five cells did not exceed 10%. Figure 4 demonstrates that the energy profiles for calcium and barium ions in the calcium channel are similar except in the region which corresponds to the outer binding site of the channel. We suppose that just this effect does determine the selectivity of the calcium channel (*see also* Discussion).

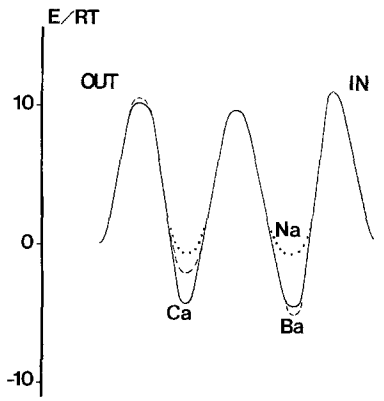


**Fig. 4.** The energy profile of calcium channel for $Ca^{2+}$, $Ba^{2+}$ and $Na^+$ ions

In a similar way we investigated the effect of lowering the pH value of the extracellular solution on the calcium channel currents. The satisfactory description of barium currents at different pH values was achieved taking $E_2(H^+) = -13.5\ RT$ and the same parameters as for calcium and barium ions. Figure 5 demonstrates that the calculated curves approximate the experimental data quite satisfactorily but there is a constant discrepancy between the theoretical and experimental data at small depolarizations, where the calculated amplitudes of the current were systematically smaller than the those found experimentally. Probably, this discrepancy is due to the use-dependent block

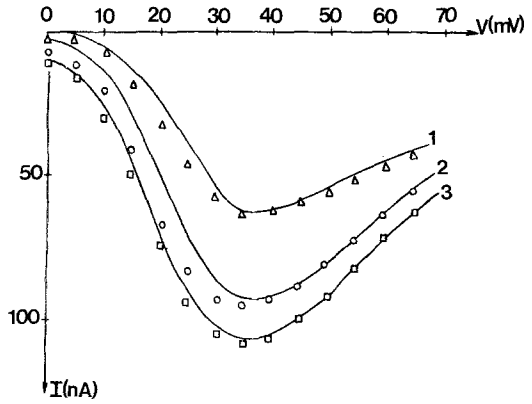


**Fig. 5.** Theoretical (curves) and experimental (points) current-voltage characteristics for barium current ($C_{out}(Ba^{2+})=30$ mM, $C_{in}=0$ at pH values equal to 5.6 (1), 6.2 (2), 7.0 (3)

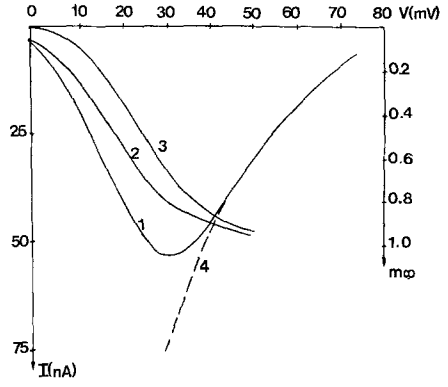


**Fig. 6.** Theoretical curves for $C_{out}(Ca^{2+})=10$ mM. (1) Stationary current-voltage characteristic; (2) $m_\infty$ for the calcium channel; (3) $m_\infty^2$ for the calcium channel; (4) current-voltage characteristic for the open calcium channel

of calcium channel by protons, i.e. at small depolarization the kinetics of channel opening becomes comparable to the kinetics of reaction between the external binding site of each channel and protons, so that in these cases an equilibrium distribution between protons located in the outer mouth of the channel and in the extracellular solution cannot be achieved and the blocking effect of protons on inward current cannot develop in a full strength.

As follows from Eq. (6), the value of the ionic current in the calcium channel is determined by two independent processes – the activation of channels and ionic transport through the open channels which have different potential-dependence. Using Eqs. (4) and (6), we have analyzed the participation of these processes in determining the ionic current at different testing potentials. The calculated values of $m_\infty(V)$ and $i(C_{in}, C_{out}, V)$ are presented in Fig. 6. As

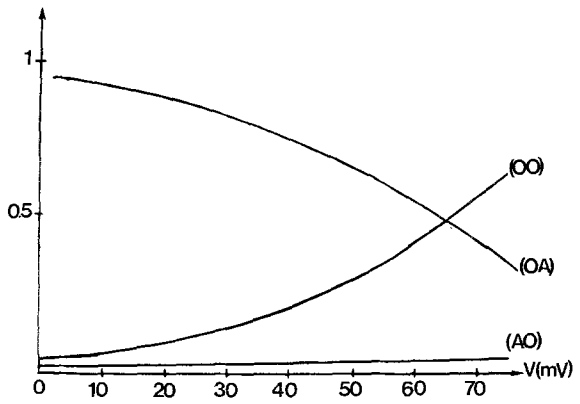


**Fig. 7.** The potential dependence of the occupation number of ion-ionic channel system ($C_{out}(Ca^{2+})=10$ mM)

can be seen from this Figure, at testing depolarization below +20 mV the amplitude of the ionic current is determined mainly by the steady-state number of open channels, whereas at testing potentials above +40 mV, by their energy profile mainly. In all experiments the concentration of carrier ions inside the cell was practically zero; that is why the current in all cases considered exponentially approached zero at large values of testing potential without reversing its direction.

The calculated potential-dependence of the occupation numbers for different states of the ion-ionic channel complex are shown in Fig. 7. From the data presented in this Figure the probability of simultaneous occurrence of two ions in the channel can be approximately estimated as the product of values $A_2$ and $A_3$. This probability does not exceed 5% when the outside concentration of penetrating ions is less than 60 mM. This estimate can be used as an argument which favors the use of a single-particle model in the present work. However, this conclusion in no way precludes the possibility that at higher concentrations of the carrier ions two ions can occupy the channel simultaneously.

## Discussion

From the data presented in Results, it follows that the main difference between calcium channel energy profiles for $Ca^{2+}$, $Ba^{2+}$ and $H^+$ ions is due to the outer binding site of the channel. So, let us consider in detail the characteristics of this binding site.

Table 2 presents the pK values for the complexes of $Ca^{2+}$, $Ba^{2+}$ and $H^+$ ions in aqueous

**Table 2.** pK values for complexes formed by $H^+$, $Ca^{2+}$, $Ba^{2+}$ ions with outer and inner binding site of calcium channel of mollusc neuronal membrane and with different functional groups of proteins in an aqueous solution taken from Martell and Smith (1977)

| Ion | $-O^-$ | $-S^-$ |  | $-COO^-$ | $-(COO^-)_2$ | $-(COO^-)_3$ | $pK_{out}$ | $pK_{in}$ |
|---|---|---|---|---|---|---|---|---|
| $H^+$ | 7.2 | 8.4 | 6.0 | 2.4 | 4.3 | 6.3 | 5.8 | – |
| $Ca^{2+}$ | 1.5 | – | – | 1.3 | 3.0 | 3.5 | 2.0 | 2.6 |
| $Ba^{2+}$ | 0.9 | – | – | 0.8 | 2.3 | 2.6 | 1.0 | 2.8 |

solution with different anionic groups of amino acids (Martell & Smith, 1977) along with pK values for the complexes of these ions with the outer ($pK_{out}$) and inner ($pK_{in}$) binding sites in the calcium channel. The pK values for dissociation of ions with functional groups of the calcium channel were calculated as follows:

$$pK = -\log K = -\log e^{-E_i} = \log e \cdot E_i \qquad (8)$$

where $E_i$ is the depth of the energy well in $RT$ units for the corresponding ion. From the comparison of the values presented in Table 2 a suggestion can be made that both the outer and inner binding sites of the calcium channel contain only one carboxylic group which strongly binds the carrier divalent ions. This conclusion is based on a fact that the value of $pK_H$ for all other groups are greater than pK values found in our calculations for the calcium channel, and the values of pK for calcium and barium ions with two or more carboxylic groups exceed our values calculated for calcium channel. However, data presented in Table 2 show that pK values calculated by us for complexes of $Ca^{2+}$, $Ba^{2+}$ and $H^+$ ions with binding sites of the calcium channel exceed the pK values for model complexes with the carboxylic group. This effect can be explained assuming the presence of some additional functional groups in the binding site of the calcium channel, which increase the stability of complexes formed in comparison with similar complexes with a single carboxylic group. Another possible explanation is that dissociation constants of complexes formed by ions increases with a decrease of the dielectric constant of the medium (Moelwyn-Hudges, 1971). The latter explanation is considered in detail in the Appendix.

Thus, there are reasons to suggest that a carboxylic group plays the main role in the process of ion binding by the site of the calcium channel; this is supported by the fact that the sequence of binding of different ions to a carboxylic group in aqueous solution ($Ni^{2+} > Co^{2+} \gtrsim La^{3+} > Cd^{2+} > Mn^{2+} > Ca^{2+} > Ba^{2+} \approx Sr^{2+}$, Martell & Smith, 1977) resembles closely the analogous sequence for the calcium channel of the mollusc somatic membrane (*see also* Akaike, Lee & Brown, 1978). It was also shown (Kostyuk & Mironov, 1982) that calcium channel in mammalian neurons possesses similar properties.

For comparison it should be mentioned that the calcium channels in nerve terminal seem to have very similar binding properties: here $H^+$ and $Ca^{2+}$ ions also compete for a common binding site with $pK \approx 6$, and the relative permeability sequence is $Ca^{2+} < Sr^{2+} < Ba^{2+}$ (Nachshen & Blaustein, 1979).

The analysis of the energy profile of the sodium channel (Hille, 1975) has shown that the active center of this channel also contains a carboxylic group. Its values of dissociation constants $pK_H = 5.3$ (Hille, 1975) and $pK_{Ca} = 1.7$ (Woodhull, 1973) are close to the values obtained in our experiments for the calcium channel. There are also some similarities in the chemical structure of the active centers of drugs which specifically block these channels. For instance, tetrodotoxin (sodium channel blocker) and niphedipin (calcium channel blocker, Fleckenstein et al., 1975) both have in their structure a N–H group bound covalently to a bulky carbohydrate residue. The derivatives of verapamil, which contain a tertiary nitrogen atom (>N) also have a similar structure. It should also be mentioned that the optical isomers of verapamil and its derivative D-600 possess stereospecificity relative to sodium and calcium channel in cardiac muscle, i.e. (–) stereoisomer blocks fast sodium channel, whereas (+)-stereoisomer selectively blocks the calcium channel (Bayer, Kalusohe, Kaufman & Manhold, 1975). However, a considerable difference

should still exist in the structure of the "selectivity filter" of the sodium and calcium channels. The "selectivity filter" in the calcium channel, contrary to the sodium channel, seems to be located in front but not behind the site of primary binding of carrier ions, as was postulated by Hille (1975) for the sodium channel. This "filter" of calcium channel prevents the monovalent cations from passing through it. The main argument in favor of this suggestion is the possibility to transfer a calcium channel into a sodium channel without changing its capability of binding other divalent cations – by introducing EGTA or EDTA in a calcium-free extracellular solution (Kostyuk & Krishtal, 1977*b*). This transformation can be interpreted as a reversible change in the structure of the outer mouth of the channel.

To summarize, we may conclude that the main difference in the mechanism of ion transport through the sodium and calcium channel is as follows: the amplitude of inward current in the sodium channel is determined mainly by the height of the energy barrier that corresponds to the central "selectivity filter" (Markin & Chizmadjev, 1974; Hille, 1975) whereas in the calcium channel it is determined by the depth of the energy hole at its external mouth. Less effective binding of $Ba^{2+}$ ions compared to that of $Ca^{2+}$ ions results in larger barium than calcium current. The binding of $Na^+$ ions by a carboxylic group is much less effective than the binding of divalent cations (Martell & Smith, 1977), and therefore the current carried by them after the modification of the external "selective filter" of the calcium channel should be considerably higher. This is actually observed in the experiments (Kostyuk & Krishtal, 1977*b*). Indeed, if we take for $K_{out}(Na^+)$ the value of 330 mM determined by Hille (1975) for the sodium channel, i.e., $E_2^{Na}=1\ RT$ (Fig. 4), and other parameters being the same as for calcium ions, the ratio between the sodium and the calcium current through single calcium channel would be equal to 16:1, which is close to the experimentally observed value 13:1 (Kostyuk, Krishtal & Pidoplichko, 1978).

Of great importance is the question about the possibility to explain on the basis of the three-barrier model of the calcium channel the experimental data about the effect of intracellular injection of $Ca^{2+}$ ions (Kostyuk & Krishtal, 1977*b*; Doroshenko & Tsyndrenko, 1978). This model predicts $pK_{Ca}$ value for the internal binding site equal to 2.6 mM (*see* Table 2), whereas the experiments show that the concentration of intracellular calcium as low as $C_{in}(Ca^{2+})=10^{-7}$ M produces a complete block of the current through the calcium channels. This discrepancy can be explained if we assume that the increase in the intracellular free calcium produces some changes in the structure of the calcium channels which make them nonconducting at such levels of $[Ca^{2+}]_{in}$ when the occupation number of the internal binding site is still far from 1.

The main arguments in favor of this suggestion come from the data about the conductance of single calcium channel. If we accept that the depth of the energy well at the inner mouth of the channel is about $14\,RT$ units (this value corresponds to $pK_{in}=8$ found in experiments with intracellular calcium injections, *see* Doroshenko & Tsyndrenko, 1978), then the value of the single-channel current calculated from Eq. (4) would be as low as $10^{-15}$ to $10^{-16}$ amps. However, the data from noise measurements of calcium channel currents give 2–3 order higher values (Kostyuk et al., 1978).

It has also been shown that the process of calcium channel inactivation depends on concentration of free $Ca^{2+}$ ions entering the cell during its activation. $Sr^{2+}$ and $Ba^{2+}$ ions produce a much weaker effect, although they carry about twice as much current through the membrane. The mechanism of this inactivation is still unknown, but there can be two alternative hypotheses: *a*) free $Ca^{2+}$ ions present inside the cell in concentration ranging from $10^{-7}$ to $10^{-8}$ M can react directly with some high-affinity binding site of the calcium channel. This process leads to the transition from the conducting state of the calcium channel to a nonconducting one. *b*) The increase of intracellular concentration of $Ca^{2+}$ ions can affect the functioning state of the calcium channel through the system of cyclic nucleotides. This viewpoint is supported by Rasmussen and Goodman (1977) as well as by recent results from our laboratory (Fedulova, Kostyuk & Veselovsky, 1981).

One possible verification of the proposed suggestion about the mechanism of blocking the calcium channels by intracellular $Ca^{2+}$ ions could be a quantitative study of similar blocking produced by intracellular administration of $Sr^{2+}$ and, especially, $Ba^{2+}$ ions. The binding constant for $Ba^{2+}$ ions at the internal binding site of the calcium channels should be close to the predicted value of about 1.5 mM.

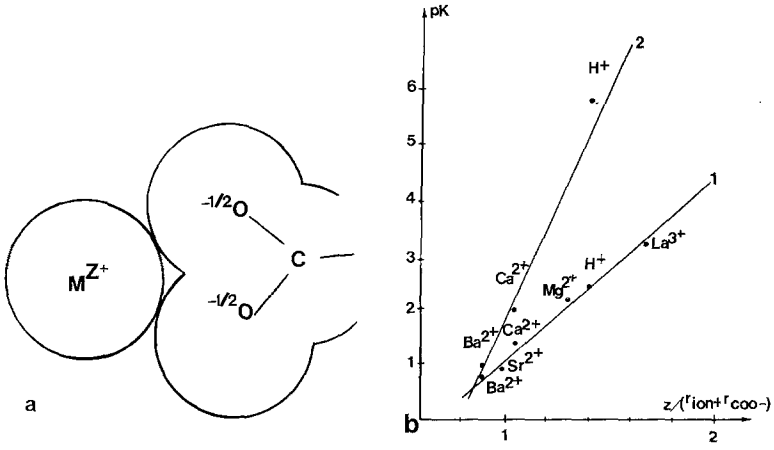


**Fig. 8.** (*a*) Schematic description of the complex of cation $M^{z+}$ and the carboxylic group. (*b*) Dependence of pK values for different ions with carboxylic group on the value of ionic radius. 1 – The aqueous solution (data from Martell & Smith, 1977); 2 – calcium channel

## Appendix

Here we shall consider the effect of dielectric constant of the calcium channel on the value of dissociation constants for complexes formed by cation and the carboxylic functional group of this channel. Let us suppose that this complex has the structure shown in Fig. 8*a*. The change in free energy $\Delta G$ of an ion during its transfer from the aqueous phase to the calcium channel, where it can form the corresponding complex with the carboxylic group, may be written in the following way:

$$\Delta G = G_1 - G_0 - \frac{z_i}{\varepsilon(r_i + r_{coo^-})} \tag{A1}$$

where $\varepsilon$ is the dielectric constant of the channel, $G_0$ and $G_1$ are the free energies of the ion in an aqueous solution and in the channel, respectively; and the last term corresponds to the energy of electrostatic interaction between the ion having charge $z_i$ and radius $r_i$ and two oxygens of the carboxylic group, which both have charge $-0.5$ and their radius is equal to the van-der-Waals radius of oxygen, i.e. $r_{coo^-} = 0.14$ nm. According to the classical thermodynamics, the value of dissociation constant $K$ for this complex has the following relation:

$$K = \exp(-\Delta G/RT). \tag{A2}$$

Thus, if $G_1$ is approximately the same for different ions (some support for this assumption comes from the data shown in Fig. 4; there we see that the heights of potential barriers in the calcium channel are nearly the same for $Ca^{2+}$, $Ba^{2+}$, $Na^+$ and $H^+$ ions), then from (A1) and (A2) we obtain

$$\mathrm{pK} = \mathrm{pK}_o + \frac{z_i \cdot \log e}{\varepsilon RT(r_i + r_{\mathrm{Coo}^-})} \tag{A3}$$

where $\mathrm{pK} = -\log K$ and $\mathrm{pK}_o = \sqrt{\log e \cdot (G_1 - G_0)/RT}$.

Figure 8*b* illustrates the dependence of pK values for complexes of different cations with the carboxylic group on the value of $z_i/(r_i + r_{coo^-})$. This Figure shows that there exists a good correlation for pK values of complexes in the aqueous solution and the radius of cation. Plotting similar data obtained for the calcium channel, we see that they can be described on the basis of Eq. (A3). The parameters in this Equation have the following numerical values: $\varepsilon = 20$ and $(G_1 - G_0) = 13\,RT$. From Fig. 4 we see that the latter value is close to the height of potential barriers in the calcium channel. The value of $\varepsilon$ is also reasonable, because channel-forming proteins are more polarizable than lipids, which have $\varepsilon = 2$, and less polarizable than water which has $\varepsilon = 80$.

Although this model is very simplified and might be considered as strongly speculative, it explains the permeability sequence of the calcium channel. From Fig. 8*b* we can estimate the dissociation constants for cations with the calcium channel, which are not tested in the present work, e.g. $K_{Sr} = 16$ mM; $K_{Mg} = 0.06$ mM, $K_{La} = 6$ μM. These values are close to those obtained by Akaike et al. (1978). Thus, the increase of the charge of the ion and decrease of its radius lead to increase of the strength of the ion binding to the carboxylic group of the calcium channel and this effect allows us to divide above-mentioned ions into two groups: permeant ions ($Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$ and $Na^+$) for which $K_{diss} > 1$ mM and nonpermeant ions or blockers ($Mg^{2+}$, $H^+$ and $La^{3+}$) for which $K_{diss} < 1$ mM. It should also be noted that this model is applicable only to the cations whose polarizability is small. In this case their interaction energy with carboxylic group can be written as the interaction of the corresponding point charges [*see* Eq. (A1)]. This is no longer valid for transition metal cations, whose polarizability is large, and just this effect does determine their energy of interaction with the carboxylic group. That is why all transition metal cations must be effective blockers of calcium conductivity. This conclusion is confirmed by many experimental data (Kostyuk & Krishtal, 1977*a*; Akaike et al., 1978).

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