Theoretical Description of Calcium Channels in the Neuronal Membrane

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Abstract. Models of ionic transport processes and functioning of calcium channels in the membrane of neurones from molluscan and rat dorsal root ganglia were developed on the basis of direct measurements of the corresponding calcium currents. Ionic transport processes are considered taking into account the effect of surface potential of the external side of the neuronal membrane. The value of the binding constant for calcium ions to negatively charged groups producing this potential is evaluated as $K_{Ca} = 70 - 90$ l/mol. The magnitude of the calcium current through the channel is determined mainly by the interaction energy of the corresponding cations with a carboxylic group which is located near the external mouth of the calcium channels, K_{Ca} of this group being 100 l/mol for molluscan and 20 l/mol for rat dorsal root ganglia. The theoretical description of the functioning of the calcium channels takes into account that intracellular cAMP participates in maintaining these channels in the functioning state. The optimal concentration of cAMP necessary for this process is about 10^{-4}—10^{-5} mol/l. It is suggested that another high-molecular factor inside the cell is also necessary for transferring the calcium channels into the functioning state.

Key words: Models — Calcium channels — Nerve cells

Introduction

The functioning of the somatic membrane of the nerve cell is largely determined by its calcium conductance (Hagiwara 1973; Reuter 1973; Kostyuk 1980; Cheung 1980; Racker 1980). Ca^{2+} ions entering the cell through the calcium channels play an important role in many intracellular processes such as the control of transmembrane (Racker 1980) and axoplasmic transport (Llinás 1979), regulation of reception and processing of information through the system of cyclic nucleotides (Cheung 1980; Greengard 1978), and even the control of protein synthesis (Llinás 1979).

This unique role of Ca^{2+} ions as activators and regulators of biological processes is related to their physico-chemical properties which are the most optimal

for their interaction with high molecular weight bioorganic substances (Kretzinger and Nelson 1976; Carafoli and Crompton 1978).

Taking into consideration the above mentioned facts, it was important to develop theoretical models of processes occuring during penetration of calcium ions through the membrane.

The present work deals with the processes of ionic transport in calcium channels of the somatic membrane of neurones from molluscan and rat dorsal root ganglia which are described on the basis of the three-barrier model of their energy profile (Part II). Since all these processes are largely influenced by the potential of the external surface of the membrane, the value of which is, in turn, determined by the extracellular concentration of Ca^{2+}, we began from the investigation of the surface potential of the neuronal membrane (Part I). Experimental data obtained by Fedulova et al. (1981) indicate that the functioning of calcium channels of the membrane is closely related to the cyclic nucleotide system inside the cell. From theoretical description of these processes presented in part III it appears that the optimal cAMP concentration necessary to keep calcium channels in the active state amounts to 10^{-4}—10^{-5} mol/l; moreover, an additional high-molecular weight factor must be present inside the cell in order to keep calcium channels in the functioning state (Part III).

I. External surface potential of the somatic membrane

It was shown earlier (Kostyuk et al. 1981; Kostyuk and Mironov 1982) that the outer surface of the somatic membrane possesses a negative fixed charge which indicates that the value of local ion concentration near the membrane (and near the ionic channels) must be larger than in the bulk solution, and the experimentally measured value of potential drop between solutions bathing the membrane must also include the value of surface potential.

Table 1. Calculated values of surface charge density σ_o and binding constants of cations to negatively charged groups of the outer membrane surface for molluscan (I) and rat dorsal root (II) ganglion neurones. The characteristics of surface groups are obtained on the basis of the shifts of current-voltage relationships to the corresponding currents.

	$\sigma_o/C.m^{-2}$	pK_H^*	K_{Ca}^*/l/mol	K_{Sr}^*/l/mol	K_{Ba}^*/l/mol
Sodium channels I	$(2.7\pm0.2)\times10^{-2}$	6.2 ± 0.2	90 ± 10	60 ± 10	23 ± 5
Calcium channels	$(3.7\pm0.2)\times10^{-2}$	6.2 ± 0.2	70 ± 10	20 ± 5	13 ± 5
II Calcium channels	$(2.4\pm0.2)\times10^{-2}$	—	70 ± 10	—	—

$pK_H = \lg K_H$

*$K_{M^{2+}}$ values are apparent binding constants corresponding to pH = 7.3

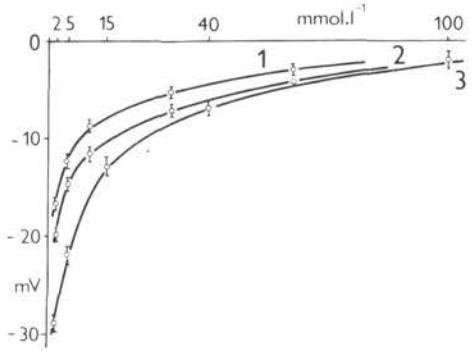

Fig. 1. Dependence of the surface potential φ on extracellular Ca^{2+} concentration for sodium (1) and calcium (2) channels in the molluscan somatic membrane and for calcium channels (3) in rat somatic membrane. Solid curves approximating the experimental points were drawn according to equation (B5) with constants taken from Table 1. Vertical bars show the experimental errors.
Composition of the starting ionic solutions:
1 — NaCl — 150 mmol/l: $MgCl_2$ — 0
2 — NaCl — 0; Tris-Cl — 170 mmol/l
3 — $MgCl_2$ — 2 mmol/l; Tris-Cl — 150 mmol/l
Ionic strength of solutions in the presence of divalent ions was kept constant by the appropriate change of Tris-Cl concentration.

On the basis of the model described briefly in Appendix A, we investigated the action of divalent ions and protons on the surface potential of the outer side of the somatic membranes. Some characteristics of this process are listed in Table 1. The data indicate that the values of σ and K_M for negatively charged groups located near the sodium and calcium channels are similar. The calculated dependences of φ on $[Ca^{2+}]_{out}$ are also similar (Fig. 1). Hence it follows that the distribution of negatively charged groups on the surface of the neuronal membrane can be considered as homogeneous and their binding properties as similar.

It should be noted, however, that the presented values of Ca^{2+}—binding constants considerably exceed those obtained for other biological membranes ($K_{Ca} = 0.1$—0.5 l/mol, — Hille et al. 1975; Ohmori and Yoshii 1977). This discrepancy may be the result of different methods used for the determination of K_{Ca} value. Since the equation (A5) used for our calculations of the surface potential includes K_{Ca} in the form $K_{Ca} \exp(-2\varphi F/RT)$, it is reasonable to compare these values, but not K_{Ca}. It can be shown (Kostyuk and Mironov 1982) that using at first the Gouy — Chapman equation (A1) for the treatment of experimental results and than correcting the calculated dependence of surface potential φ on $[Ca^{2+}]_{out}$ by taking into account the Ca^{2+} binding (i.e. the procedure employed by Hille et al. (1975) and Ohmori and Yoshii (1977)), one obtains the values of φ which are

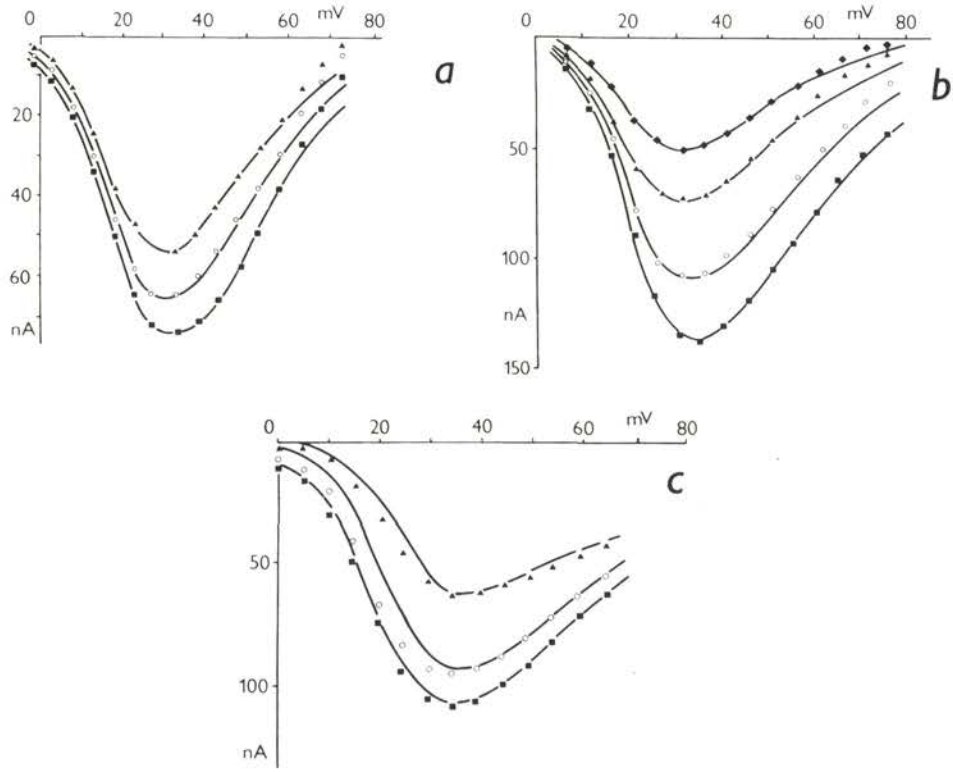

Fig. 2. Theoretical approximation of experimental current-voltage relationships for molluscan somatic membrane obtained for $[Ca^{2+}]_{in}=0$.
a — calcium current; $[Ca^{2+}]_{out}=10$ (▲); 30 (○); 60 (■) mmol/l.
b — barium current; $[Ba^{2+}]_{out}=5$ (■); 10 (▲); 30 (○); 60 (■) mmol/l.
c — barium current at different pH = 7.0 (■); 6.2 (○); 5.6 (▲); $[Ba^{2+}]_{out}=30$ mmol/l.

30—40 mV larger than in the case of simultaneous determination of σ, K_{Ca} and φ (see Appendix A and Kostyuk and Mironov 1982). Hence the difference between the values of K_{Ca} exp $(-2\varphi F/RT)$ thus calculated and those obtained by Hille et al. (1975) and Ohmori and Yoshii (1977) is much smaller than in the case of K_{Ca} values.

Because the value of surface potential in both cases could not be measured directly, and only its change was determined depending on the composition of extracellular solution, additional data are necessary for finding out which way of description of dependence on $[Ca^{2+}]_{out}$ is correct. The results of direct experimental determination of cation-binding constants for phospholipid vesicles and measurement of their surface potential (ξ-potential) (Nir et al. 1978; Hammoudan

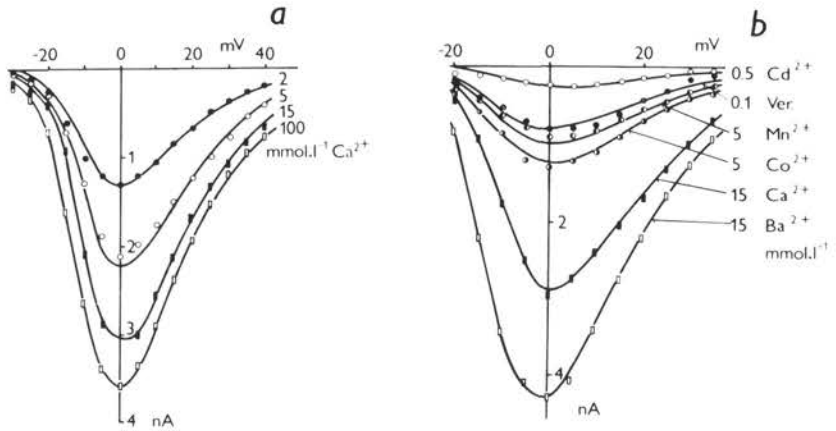

Fig. 3. Theoretical approximation of experimental current-voltage relationships for calcium channels of rat somatic membrane obtained for $[Ca^{2+}]_{in} = 0$.
a — calcium current; $[Ca^{2+}]_{out} = 2$ (●); 5 (○); 15 (■); 100 (□) mmol/l.
b — barium current (□); $[Ba^{2+}]_{out} = 15$ mmol/l (□); calcium current, $[Ca^{2+}]_{out} = 15$ mmol/l (■) and additions of $[Co^{2+}]_{out} = 5$ mmol/l (◑), $[Mn^{2+}]_{out} = 5$ mmol/l (◑), [verapamil]$_{out} = 0.1$ mmol/l (●), $[Cd^{2+}]_{out} = 0.5$ mmol/l (○).

et al. 1981; Ohki and Kurland 1981) can be taken as a possible independent criterion. These experiments give the values of K_{Ca} equal to 35 l/mol (Nir et al. 1978), 75 l/mol (Hammoudan et al. 1981) and 30 l/mol (Ohki and Kurland 1981) and are comparable with the values calculated by us for the external surface of the somatic membranes.

The calculations of external surface potential for somatic membranes were used for adjusting the current-voltage curves for calcium currents along the potential axis, and to obtain the values of near-membrane concentration of carrier ions near the external mouth of the calcium channels according to the equation (A3).

II. Ionic transport processes in calcium channels

Our theoretical description of ionic transport processes in the calcium channel was based on a three-barrier model of its energy profile described briefly in Appendix B. Figs. 2 and 3 demonstrate the current-voltage relationships for molluscan ganglion neurones and for rat dorsal root ganglion neurones respectively. The current-voltage relationships shown in Figs. 2 and 3 were plotted taking into account the corresponding value of the external surface potential, i.e. all experimental curves are shifted to the right by the value $|\varphi|$ determined for a given value

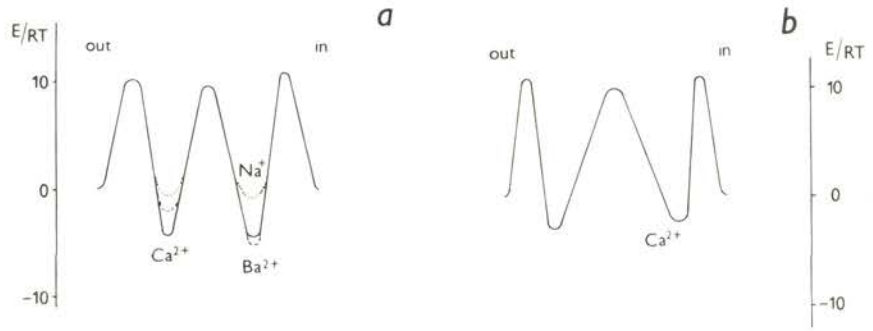

Fig. 4. Calculated energy profile of calcium channel for molluscan (a) and rat (b) somatic membranes for Ca^{2+} (———), Ba^{2+} (— — —) and Na^{+}(...) ions.

of $[Ca^{2+}]_{out}$. From the comparison of theoretical and experimental curves it can be concluded that the three-barrier model for the energy profile of the calcium channel gives an adequate description of the ionic transport processes in this channel.

Fig. 4 presents the calculated energy profiles for calcium ions in the calcium channels of molluscan and rat dorsal root ganglion neurones. It is important to note that the height of the potential barriers for calcium ions is approximately the same and in both species of neurones their values are close to 11—13 RT obtained by Hille for sodium ions in the sodium channel of the frog Ranvier node (Hille 1975) and by Urban et al. (1980) for sodium ions in the gramicidin channel.

Some characteristics of the external binding site in the calcium channels are listed in Table 2. It follows from these data and the corresponding current-voltage curves (Figs. 2 and 3) that the current value in the calcium channel is determined mainly by the affinity of carrier ions to the external binding site. When the interaction energy between the ion and the binding site is larger, i.e. when the energy well is deeper (Table 2), the permeability of the corresponding ion decreases and its blocking capability increases (Fig. 3).

Data presented in Table 2 also show that the external binding site of the calcium channel of the molluscan somatic membrane has larger affinity to cations than that of rat dorsal root ganglion neurones. It can be assumed that the external binding site contains only one carboxylic group (Kostyuk et al. 1981; Kostyuk and Mironov 1982). This assumption can be made because pK_H values for all other groups present in proteins are higher than 6, and pK values for complexes of Ca^{2+} and Ba^{2+} ions with two and more carboxylic groups (Martell and Smith 1977) exceed pK values calculated for the calcium channel of molluscan ganglion neurones.

These qualitative arguments can be put in a quantitative form. Let us consider

Table 2. Some characteristics of the outer cation-binding site of the calcium channel in the somatic membrane of molluscan (I) and rat dorsal root (II) ganglion neurones compared with binding by glycine. E is the energy of potential well in RT units corresponding to the outer binding site of the calcium channel, $pK = \log K = -E \log e$.

	Ion	−E	K/l/mol	pK	pK* glycine
I		2.3	11	1.0	
	Ba^{2+}				0.8
II		1.5	4.5	0.65	
I		4.6	100	2.0	
	Ca^{2+}				1.4
II		3.0	20	1.3	
II	Co^{2+}	5.8	320	2.5	5.0
II	Mn^{2+}	6.0	400	2.6	3.2
I		11.5	10^5	5.0	
	Cd^{2+}				4.8
II		9.8	1.8×10^4	4.3	
II	Verapamil	9.9	2×10^4	4.3	—
I	H^+	13.6	6.2×10^5	5.8	2.4

*Martell and Smith 1977

the spatial arrangement of the complex cation—carboxylic group has a structure depicted schematically in Fig. 5a. The radius of oxygen atoms in the carboxylic group is taken as their van-der-Waals radius ($r_{coo^-} = 0.14$ nm), and the radius of the cation is taken according to Pauling's data (Pauling 1974). pK for the dissociation of this complex can be expressed in the following way (Moelwyn—Hughes 1971):

$$pK = pK_o + \frac{z_i . \log e}{4\pi\varepsilon_o\varepsilon_r R T (r_i + r_{coo^-})} \quad (1)$$

where z_i is the charge of the ion, ε_o is the permittivity of free space, ε_r is the dielectric constant of the medium, 10^{pK_o} is the hypothetical binding constant for the complex when $z_i = 0$.

Fig. 5b illustrates the dependence of pK values for complexes of glycine with different cations in aqueous solutions (Martell and Smith 1977) on $z_i(r_i + r_{coo^-})$ value. The figure shows a good correlation between pK and the radius of the cation. The ploting of a similar dependence for the calcium channel of molluscan somatic membrane indicates that dielectric constant near the outer binding site of the calcium channel should be taken four times smaller than that for water. From the data presented in Fig. 5b we can also estimate the probable value of pK for complexes of Sr^{2+}, Mg^{2+} and La^{3+} with the outer binding site of the calcium channel ($K_{Sr} = 16$ mmol/l; $K_{Mg} = 0.06$ mmol/l; $K_{La} = 6$ μmol/l). In the case of transition

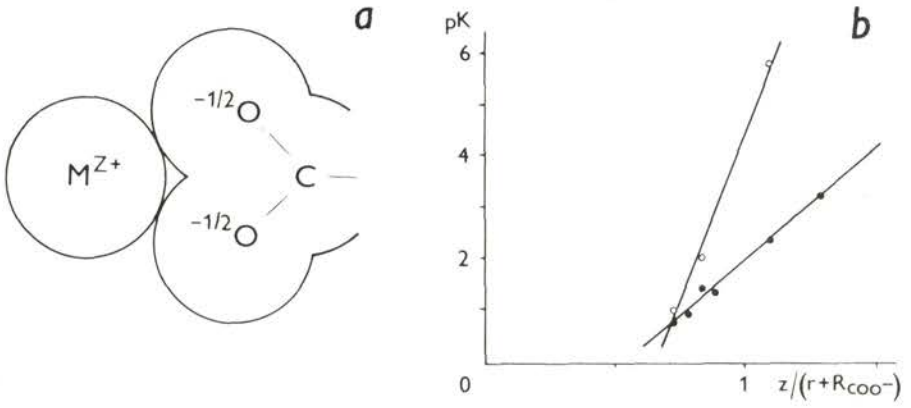

Fig. 5 a — schematic description of the complex of M^{2+} cation and carboxylic group. **b** — dependence of pK for complexes of cations with glycine in aqueous solutions (1) and with the outer binding site of the calcium channel of molluscan somatic membrane (2) on $z_M/(r_{ion}+r_{coo^-})$. Every point corresponds to a certain ion.

metals we cannot use expression (1), because the interaction of these cations with different anions is not a purely electrostatic one. However, calculated values of pK for complexes of the transition ions with the external binding site of the rat somatic membrane are close to the values for the corresponding complexes with glycine (Table 2); so, we may assume that in this case the external binding site also contains only one carboxylic group.

Woodhull (1973) and Hille (1975) have shown that the cation-binding site of the sodium channel also contains a carboxylic group. It is characterized by values of $pK_H = 5.3$ and $pK_{Ca} = 1.7$ which are close to those calculated for the calcium channels of molluscan somatic membrane (Table 2).

It is known (Kostyuk and Krishtal 1977) that the calcium channels of the somatic membrane of molluscan neurones can be transformed into sodium channels by the introduction of EDTA into calcium-free extracellular solution. In this case the following approximate relations between maximal currents through single channels are observed $i_{Na} : i_{Ca} : i_{Ba} = 13 : 2 : 1$ (Kostyuk et al. 1978). If we assume that the value of the energy well for sodium ions in the EDTA-modified calcium channel equals $1RT$ (Hille 1975), and the potential barriers are the same as for Ca^{2+} ions (Fig. 4a) then the ratio of calculated maximal single channel currents will be $i_{Na} : i_{Ba} : i_{Ca} = 16 : 2 : 1$ agreeing well with the experimental data (Kostyuk et al. 1978). It should also be noted that the selectivity for such modified calcium channels regarding monovalent cations is nearly the same as for the sodium channels in the somatic membrane (Kostyuk and Shuba 1982).

Thus, taking into consideration all the above-mentioned facts it can the

assumed that the ion transport in the sodium and calcium channels is determined by chemical groups similar in their chemical and spatial structure. However, although Ca^{2+} ions can pass through the sodium channel (Woodhull 1973), sodium ions pass through the calcium channel only in the case of its modification by EDTA (Kostyuk and Krishtal 1977; Kostyuk and Shuba 1982). In our opinion, such a modification is related to EDTA-mediated removal of free Ca^{2+} ions from the extracellular solution. It could be suggested that in this case a loss of Ca^{2+} ions takes place also from the external and internal binding sites of the calcium channel, thus setting the way free for the passage of monovalent ions through the channel. A complication for such an explanation arises from the fact that the concentration of free Ca^{2+} ions in commercial reagents and distilled water does not actually exceed 10^{-4} mol/l. According to estimated data of the dissociation constants for the complex of Ca^{2+} ions with the binding sites of the calcium channel (Kostyuk et al. 1981), the relative number of calcium channels containing Ca^{2+} ions should be a few percents only and a noticeable sodium current has to be expected even without the addition of EDTA. Therefore, to explain the effect of EDTA on the calcium channel we must assume that it contains one more binding site for Ca^{2+} ions accessible for the ions present only in the extracellular solution. This binding site cannot be situated on the ionic transport pathway and should have a high affinity to Ca^{2+} ions, its K_{diss} being about 10^{-6} mol/l. This value is typical for different Ca^{2+}-binding proteins, such as calmodulin, troponin C, parvalbumin (Kretzinger and Nelson 1976).

It is also of great interest whether the proposed model describes adequately the experimental results obtained with intracellular introduction of Ca^{2+} ions (Kostyuk and Krishtal 1977; Doroshenko and Tsyndrenko 1978). It follows from the model calculations (Kostyuk et al. 1981) that the pK value for the internal binding site of Ca^{2+} ions in the calcium channel of molluscan somatic membrane must be equal to 2.5, while experiments show (Kostyuk and Krishtal 1977; Doroshenko and Tsyndrenko 1978) that the calcium current is completely suppressed when $[Ca^{2+}]_{in} = 10^{-7}$ mol/l. Such a discrepancy between theoretical and experimental data can be explained by assuming that the intracellular injection of Ca^{2+} ions has some specific inactivating effect on the calcium channel even when $[Ca^{2+}]_{in}$ is so small that the occupancy of the internal binding site in the channel is much less than unity.

The main argument in favour of this suggestion results from the data on the single calcium channel conductance. If we accept that the depth of the energy well corresponding to the internal binding site in the calcium channel is 14RT and $pK_{in} = 8$ (Doroshenko and Tsyndrenko 1978), then the value of the single channel current calculated from equation (B4) would be as low as 10^{-15}—10^{-16} A. The data obtained from noise measurements of the calcium channel, as well as from our model give 2—3 order higher values (Kostyuk et al. 1978).

This suggestion is also supported by the experimental data (Eckert and

Tillotson 1981; Doroshenko et al. 1982) which show that the calcium channel inactivation depends on the amount of intracellular Ca^{2+} entering the nerve cell during excitation but not on the value of transmembrane potential. At present the mechanism of this inactivation is still unknown, though one may suppose that it is due to direct interaction of Ca^{2+} ions with proteins forming the ionic channel, or to their indirect effect through cyclic nucleotide metabolism (see below).

III. Interaction of calcium channels with cyclic nucleotide metabolism

One of the features of calcium conductance in the somatic membrane of nerve cells distinguishing it from other types of ionic conductances is a progressive decrease of its value during cell perfusion (Kostyuk 1980; Fedulova et al. 1981). In this connection it was suggested (Kostyuk 1980) that the functioning of calcium channels is more closely related to intracellular metabolic systems than that of sodium and potassium channels (Kostyuk 1980; Kostyuk and Mironov 1982).

Experiments (Fedulova et al. 1981) performed on rat dorsal root ganglion neurones demonstrated that the intracellular injection of cAMP together with ATP and Mg^{2+} ions leads to restoration of calcium conductance which is usually rapidly declining in the course of intracellular perfusion with saline free from these substances. Therefore, one may suppose that the main reason for the decline of calcium conductance during intracellular perfusion is the breakdown of the cyclic nucleotide metabolism due mainly to washing out of cAMP from the cell. If we suppose that this process, as well as the process of restoration of cAMP inside the cell can be described by simple diffusion, a quantitative model of these processes can be proposed (Appendix C).

This model predicts an abrupt decrease of cAMP concentration during intracellular perfusion and its slow restoration near the active centres, which regulate the conductance of calcium channels during intracellular application of this substance.

Experimental data indicate that if the calcium current during intracellular perfusion is decreased to zero, it cannot be restored by intracellular application of cAMP. Furthermore, the restoration of calcium current during intracellular application of cAMP is always followed by its subsequent decrease which is, however, much slower than in the case of perfusion with cAMP-free solution. These facts allowed us to suggest that in the course of perfusion besides cAMP, an unknown factor X is washed out of the cell. Let us suppose that the sequence of reactions has the form:

$$X + cAMP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Y \overset{k}{\rightarrow} \ldots \qquad (2)$$

then the stationary concentration [Y] can be expressed by the Michaelis—Menten

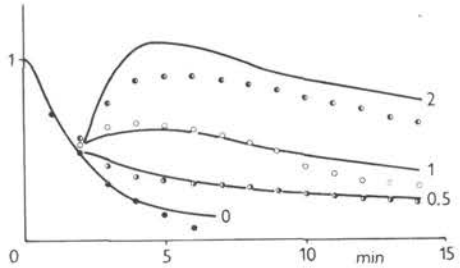

Fig. 6. Calculated time-dependence of calcium current for rat somatic membrane. The magnitude of I_{Ca} for $t=0$ was taken as unity. The value of $cAMP_{perf}/cAMP_{in}$ assumed in calculations is shown near the corresponding curve. Points indicate experimental data taken from (Fedulova et al. 1981). The moment of cAMP injection is indicated by arrow.

equation (Volkenstein 1975):

$$[Y] = k_1[X]\,[cAMP] \,/\, (k_2 + k_{-1} + k_1[cAMP]) \qquad (3)$$

On condition that the first stage is the slowest one (i.e. $k_2 + k_{-1} > k_1\,[cAMP]$), we obtain that [Y] and, hence, the number of functioning calcium channels is proportional to the product of Y and cAMP concentrations inside the cell. Their changes at any moment from the start of cell perfusion can be described by equation (C1), and the changes of cAMP concentration inside the cell after its introduction in the perfusing solution by equation (C2).

Thus, the proposed model contains 3 parameters — $\alpha_{cAMP} = x_o/\sqrt{2D_{cAMP}}$, $\alpha_X = x_o/\sqrt{2D_X}$ and $[cAMP]_{perf} \,/\, [cAMP]_{in}$ (Appendix C). The values of parameters α_{cAMP} and α_X were obtained by approximation of the experimental data on the time-dependence of the calcium current decline in the case of intracellular perfusion by cAMP-free solution.

Fig. 6 shows the theoretical time-dependences of the calcium current decline calculated for different assumed relations $[cAMP]_{perf} \,/\, [cAMP]_{in}$. The latter shows how many times the cAMP concentration inside the cell during perfusion $[cAMP]_{perf}$ is larger than the unknown initial cAMP intracellular concentration $[cAMP]_{in}$. Experimental data shown in Fig. 6 by points were obtained for different cells, whereas cAMP concentration in the perfusing solution was equal to 5×10^{-5} mol/l.

Since the theoretical curves for different values of $[cAMP]_{perf} \,/\, [cAMP]_{in}$ give a satisfactory description of the experimental results obtained for different cells, we can conclude that the cAMP concentration necessary for the maintenance of calcium conductance in the functioning state lies in the range of 10^{-4}—10^{-5} mol/l.

The ratio of model parameters α_{cAMP} and α_X is equal to $\sqrt{D_x/D_{cAMP}}$. The calculated values of α_{cAMP} and α_X vary from cell to cell, but their ratio being approximately constant and equal to 3 ± 1. From this we can anticipate that the diffusion coefficient of the factor X has a 5—10 times smaller value than the diffusion coefficient of cAMP and, hence, this substance has a considerably higher molecular weight than the cAMP molecule.

At present, we know at least several high-molecular weight substances (Greengard 1978; Cheung 1980) taking part in the intracellular metabolism of cAMP. One of these substances can be linked to the maintenance of the calcium permeability of the cell membrane. However, more data are needed to identify this substance and the mechanism of its action.

Appendix A. *Calculations of the surface potential.*

According to the theory of Gouy and Chapman, the expression connecting surface potential φ, surface charge density σ and composition of extracellular solution has the following form:

$$\sigma = \pm \sqrt{2\varepsilon_o \varepsilon_r R T \sum_i [M_i]_{out} (\exp(-z_i \varphi F/RT) - 1)} \tag{A1}$$

where ε_r is the dielectric constant of the aqueous phase, ε_o is the permittivity of free space, $[M_i]_{out}$ is the bulk concentration for ion i, and z_i is its charge.

The ions in the solution can, however, form complexes with negatively charged groups located on the membrane surface thus changing the value of σ. If these complexes are neutral (experiments made on phospholipid membranes (Nir et al. 1978) do support this assumption), the expression for the modification of the surface charge density will be described by Langmuir's isotherm:

$$\sigma = \sigma_o / (1 + \sum_i K_{M_i} [M_i]) \tag{A2}$$

where σ_o is the value of surface charge density when all surface groups are free, K_{M_i} is the binding constant for ion i, and

$$[M_i] = [M_i]_{out} \exp(-z_i \varphi F/RT) \tag{A3}$$

is the near-membrane concentration of binding ion i.

If we take into consideration that the binding constants of divalent cations and protons to different anions are much higher than those of monovalent ions (Martell and Smith 1977), the equation (A2) can be rewritten as follows:

$$\sigma = \frac{\sigma_o}{1 + K_{Ca}[Ca^{2+}] + K_H[H^+]} = \frac{\sigma_o'}{1 + K_{Ca}'[Ca^{2+}]} \tag{A4}$$

where $\sigma_o' = \sigma_o/(1 + K_H[H^+])$ is the apparent surface charge density and $K_{Ca}' = K_{Ca}/(1 + K_H[H^+])$ is the apparent binding constant for Ca^{2+} ions for a given value of pH.

Thus, from equations (A1, A3, A4) the following basic equation can be obtained:

$$|\sigma_o'|/(1 + K_{Ca}'[Ca^{2+}]_{out}e^{-2\varphi F/RT}) = \sqrt{2\varepsilon_r\varepsilon_o RT\Sigma_i[M_i]_{out}(e^{-z_i\varphi F/RT} - 1)} \quad \text{(A5)}$$

which was used in our calculations.

The change in the value of surface potential $\Delta\varphi$ depending on the composition of extracellular solution was determined from the shift of the falling phase of the current-voltage curve (Kostyuk et al. 1981; Kostyuk and Mironov 1982). The values of surface potential were obtained by adding an arbitrary constant φ_o to the value of $\Delta\varphi$. Since the dependence of φ on $[Ca^{2+}]_{out}$ is nonlinear, the values of parameters K_{Ca}', σ_o' and φ_o were found by minimization of mean square deviations of calculated shifts (according to equation A5) from the experimental ones.

Appendix B. *Calculation of single channel current using a three-barrier model of the calcium channel energy profile.*

According to the theory of ionic transport processes (Markin and Chizmadzhev 1974; Hille 1975), the stationary current through the ionic channel is determined by constants characterizing the ion transfer through the potential barriers. If we impose an electric field across the membrane, the expressions for these constants have the form:

$$\begin{aligned} k_1 &= a_1[Ca^{2+}]_{out}(-E_1 - \alpha\psi) & k_{-1} &= a_{-1}\exp(-E_1 + E_2 + \alpha\psi) \\ k_2 &= a_2\exp(-E_3 + E_2 - \beta\psi) & k_{-2} &= a_{-2}\exp(-E_3 + E_4 + \beta\psi) \\ k_3 &= a_3\exp(-E_5 + E_4 - \gamma\psi) & k_{-3} &= a_{-3}[Ca^{2+}]_{in}\exp(-E_5 + \gamma\psi) \end{aligned} \quad \text{(B1)}$$

where a_i are the pre-exponential constants, E_i — the dimensionless extremal energies of the interaction of the ion with the ionic channel in RT units (Fig. 7a); α, β, γ are the relative transmembrane potential drops on the corresponding potential barriers (we also assume that they must satisfy the equation $\alpha + \beta + \gamma = 1$), $[Ca^{2+}]_{out}$ and $[Ca^{2+}]_{in}$ are the local concentrations of carrier ions near the outer and inner mouth of the channel respectively, ψ is the dimensionless potential in RT/F units.

In order to decrease the number of parameters in the model, we assumed that all a_i are equal, and the outer and inner potential barriers are located symmetrically.

The stationary current through the calcium channel was calculated using the diagram method (see, e.g. Markin and Chizmadzhev 1974; Hille 1975). The

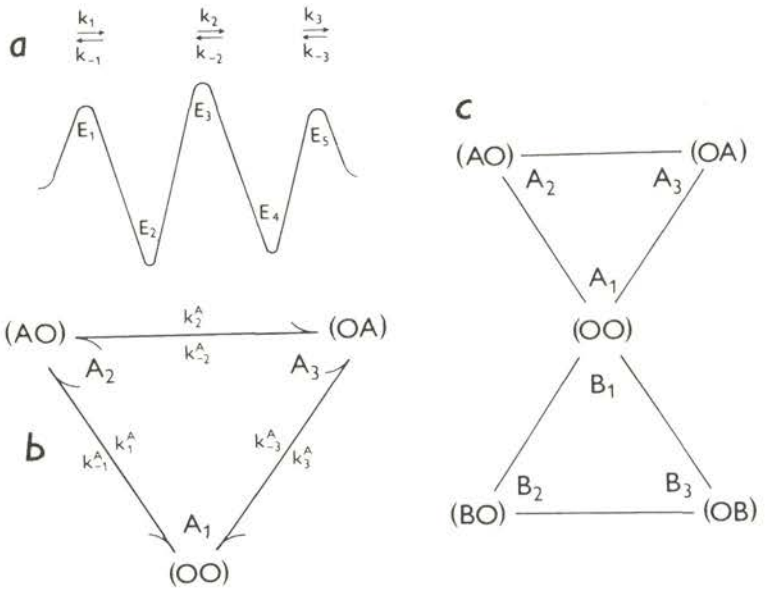

Fig. 7. Schematic description of the energy profile for a three-barrier model (a) and diagram of states of the ion-ionic channel complex for the case of the presence of one type (b) and two types (c) of ions in the bathing solutions. Indices A_i and B_i correspond to the state of ionic channel, e.g. $A_2 \equiv (A0)$ is the state when the outer potential well (see Fig. 7a) is occupied by the ion and the inner potential well is empty.

diagrams of the states of the ion-ionic channel system are shown in Figs. 7b and 7c for cases when only one permeant ion or permeant ions of two types are present in the bathing solutions, respectively. The occupation numbers A_i and B_i (Fig. 7) can be expressed by the ion transition rate constants through the potential barriers in the following way, e.g.

$$X_1 = k^x_{-1}\, k^x_2 + k^x_2\, k^x_3 + k^x_{-1}\, k^x_3 \tag{B2}$$

where $X = A,B$.

According to the continuity principle, the value of the ionic current should be equal to the difference of two opposite currents through any of the potential barriers. Their values are determined by the product of the occupation number of the corresponding potential well and the rate constants of ion transition through the corresponding potential barrier. Thus, for the case of the diagram shown in Fig. 7b the current carried by ion *i* through a single open channel can be determined as

$$i = ze(k^A_1\, A_1 - k^A_{-1}\, A_2) \,/\, N_1 \tag{B3}$$

and of the diagram shown in Fig. 7c

$$i = ze(k^A_1\, A_1 - k^A_{-1}\, A_2)\, B_1 \,/\, N_2 \tag{B4}$$

where $N_1 = A_1 + A_2 + A_3$ and $N_2 = A_1B_1 + B_1(A_2 + A_3) + A_1(B_2 + B_3)$ are normalization constants.

The expression of the stationary current has the following form:

$$I = P\, m_\infty^2(V)\, i([Ca^{2+}]_{out}, [Ca^{2+}]_{in}, V) \tag{B5}$$

where P is the coefficient of proportionality, $m_\infty^2(V)$ is the portion of channels open at potential V, $i([Ca^{2+}]_{out}, [Ca^{2+}]_{in}, V)$ the value of single channel current calculated according to (B3) or (B4). The voltage dependence of $m_\infty^2(V)$ was taken as

$$m_\infty(V) = [1 + \exp(-b(V - V_{1/2}))]^{-1} \tag{B6}$$

where the values of the steepness parameter b and the potential $V_{1/2}$ were the same for all current-voltage relationships.

The model parameters of the energy profile of the calcium channel for the carrier ions (Ca^{2+}, Ba^{2+}) were determined by minimization of the mean-square deviation of the calculated current-voltage relationship taken in the form of equation (B5) from the experimental one. The model parameters for ion blockers (H^+, Cd^{2+}, Mn^{2+}, Co^{2+} and verapamil) were chosen in the same way as for Ca^{2+} ions, except for the depth of the first (outer) potential well (Fig. 7a) which corresponds to the outer cation-binding site in the calcium channel.

From equations (B1—B5) it follows that an increase of all potential barriers by the same value ΔE will lead to an $\exp(\Delta E)$-fold decrease of the current calculated by equation (B5). Since this transformation will change only the coefficient of proportionality P (eq. B5), the true values of potential barriers can be estimated only from the results of single channel conductance measurements. Taking these experimental data for our model analysis we obtain that $\Delta E = 5RT$ for molluscan somatic membrane. Since such experiments were not performed for the rat somatic membrane, in order to determine the true energy profile for the calcium channel in this object we used the same value ΔE.

Appendix C. *The model of intracellular linear diffusion of cAMP (Kononenko and Mironov 1980; Mironov 1982).*

Let us assume for simplicity that the cell is a linear system, the perfusion pore is placed at the origin of coordinates and the value of the calcium current is determined by diffusion processes of cAMP molecules inside the cell, and their interaction with a certain active centre located at a distance x_0 from the perfusion pore. Furthermore, we assume that the characteristic time for the reaction of cAMP molecules with the active centre is less than the characteristic time of cAMP diffusion inside the cell. This assumption can be substantiated if we compare the

rate constants for the metabolic system of cyclic nucleotides (Severin 1981) and the time parameters of the cAMP diffusion inside the cell.

If the cAMP concentration in the solution used for perfusion $[cAMP]_{perf}$ is zero and its concentration in the cell at the beginning is $[cAMP]_{in}$, then the time-dependence of the intracellular concentration during peruesion $[cAMP]_{perf}$ near the active centre is given as follows (Jeffreys and Swirls 1966):

$$[cAMP]_{perf} = [cAMP]_{in} \operatorname{erf}(x_o/\sqrt{2Dt}) \tag{C1}$$

where $\operatorname{erf}(z)$ is the error function (Jeffreys and Swirls 1966) and D is the diffusion coefficient of cAMP inside the cell.

In the case when perfusion is carried out by cAMP-containing solution, the expression for $[cAMP]_{perf}$ has the following form (Kononenko and Mironov 1980; Mironov 1982):

$$[cAMP]_{perf} = ([cAMP]_{perf} - [cAMP]_{in})\,(1 - \operatorname{erf}(x_o/\sqrt{2Dt})) + [cAMP]_{in}) \tag{C2}$$

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