Short communication

Some Predictions Concerning the Calcium Channel Model with Different Conformational States

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Earlier (Kostyuk et al. 1982), we proposed a model of the possible molecular organization of the ion-transporting pathway in the calcium channel based on the results of detailed study of its permeability using different penetrating and blocking cations as probes of the functionally important channel sites. According to this model shown in Fig. 1, the channel selectivity for divalent cations is mainly determined by the intrachannel selectivity filter which, probably, contains a single carboxylic group. Therefore transition metal cations which form strong complexes with the filter are effective blockers of calcium conductance, whereas alkaline earth metal cations do penetrate.

Later we have shown (Kostyuk et al. 1983) that the selective transport of divalent cations by calcium channels in normal physiological conditions would be determined by a different kind of interaction of Ca ions with the channel. This effect was independent on the membrane potential, and we suggested that this particular Ca binding site is located on the outer side of the membrane. On the basis of affinities of alkaline earth metal cations to this site we predicted also that its molecular structure may resemble those high-affinity Ca binding sites, observed e.g. in troponin C, calmodulin, parvalbumin etc. (Kretzinger and Nelson 1976). According to our model, the removal of Ca ions from this site induces a conformational transition of the whole channel to a state when it can pass monovalent cations.

Quite recently our data concerning the control of the calcium channel selectivity of divalent vs. monovalent ions in the micromolar range of extracellular Ca^{2+} were confirmed by Almers and McCleskey (1984) for frog skeletal muscle, by Hess and Tsien (1984) for isolated cardiomyocytes, and by Fukushima and Hagiwara (1985) for mouse myeloma cells. However, these authors proposed another model of calcium channel functioning. They assumed that the calcium channel contains only two high affinity calcium binding sites. In normal physiological conditions, Ca ions can fill both sites and interact according to the Coulomb law.

On lowering the external Ca concentration down to micromolar range some channels loose Ca ions from their interior, thus acquiring the ability to pass other (e.g. monovalent) cations. This model can describe the observed dependence of mixed current I_{Na+Ca} over a wide range of Ca concentrations from 0.01 μmol/l to 100 mmol/l including the effects of Ca blockade of monovalent ion current in the micromolar range, the absence of any current at [Ca] = 0.01 − 1 mmol/l and the saturation of calcium current at higher Ca concentrations.

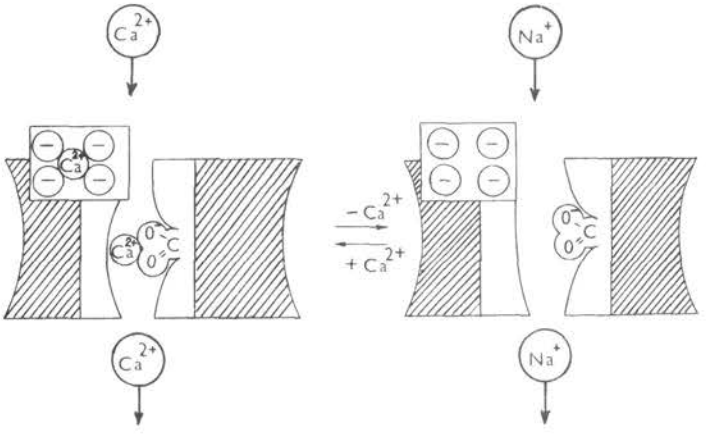

Fig. 1. Suggested Ca-binding sites in the calcium channel and their participation in its functioning (also see text).

These studies revealed also the existence of anomalous mole fraction behaviour for calcium conductance. According to the one-ion model, the amplitude of mixed Ca + Ba current must increase monotonically from one limiting value to another when the concentration of one ion changes while keeping the total concentration constant. However, the concentration dependence observed had a minimum which could be explained by the two-ions model.

Despite its attractiveness, the two-ions model contradicts a number of experimental findings. In particular, the postulated effects of the Coulomb repulsion between cations in the calcium channel were not observed for other Ca-binding proteins with high-affinity sites. Although these proteins usually contain several Ca-binding sites not distant from each other, differences between sequential pK values corresponding to their filling by Ca ions do not exceed ±0.5 units (Levine and Williams 1982). This difference is considerably smaller than predicted by the two-particle model (Δ pK = 3—4). Generally, the observed small increases in or decreases of sequential pK's are being attributed to some conformational transitions of Ca-binding proteins which play an important role in their

function and can be revealed by different spectroscopy methods. The extent of these transitions depends on the physico-chemical properties of a given ion.

Moreover, the experimental values of the exit rate constant for the high-affinity Ca binding site in proteins does not exceed $k_{off} \leqslant 10^3\ s^{-1}$ (Levine and Williams 1982) which is by 2—3 orders of magnitude smaller than the observed ion flow in the calcium channel (Kostyuk et al. 1981). This was used (Kostyuk et al. 1982; Kostyuk et al. 1983) to argue against the possible location of the high affinity Ca binding site on the ion pathway in the calcium channel.

Hess and Tsien (1984) overcame this discrepancy by lowering the heights of the terminal potential barriers. However, their low values ($E_{barrier} = 4$ RT) lead to a high entrance rate for Ca ions $k_{on} \cong 10^{11}\ mol^{-1}.l.s^{-1}$, which is considerably lower than the corresponding diffusion limit $k_{on} \cong 10^9\ mol^{-1}.l.s^{-1}$.

According to the two-ions model, the ability of Ca ions to block the monovalent ion current through the calcium channel must be approximately equal independent on the side of their application (intra- or extracellular). However, in our studies of snail neurones (Kostyuk et al. 1983) and model calcium channels induced by lathrotoxin in planar bilayer membranes (Mironov et al. 1985) the effect of Ca ions was strictly asymmetrical.

A general model of a channel with two conformational states was considered by Lauger, Stephan and Frehland (1980). However, this model is quite complicated. Therefore, we propose here a simpler model with a kinetic scheme equivalent to the mechanism of functioning of the calcium channel shown in Fig. 1

$$S'_{Na} \underset{y_{Na}}{\overset{x_{Na}}{\rightleftarrows}} S_0 \underset{a}{\overset{b}{\rightleftarrows}} S_{Ca} \underset{x_{Ca}}{\overset{y_{Ca}}{\rightleftarrows}} S'_{Ca}$$

where S_0 and S_{Ca} are two different conformational states of the empty calcium channel, differing in the occupation of the high affinity Ca channel binding site, a and b being the rate constants of transitions between them. S'_{Na} and S'_{Ca} are sodium and calcium conducting states, respectively, $x_i = k^i_1 + k^i_{-2}$, $y_i = k^i_{-1} + k^i_2$ (according to Lauger er al. (1980) for each conformational state we considered, for simplicity, the one-site one-ion model, where k^i_j and k^i_{-j} stand for rate constants of jumps of i-th ion across the j-th potential barrer in the forward and backward directions). $S_0 \leftrightharpoons S'_{Na}$ and $S_{Ca} \leftrightharpoons$ participate in Na and Ca transport through the channel. Solving the system of linear equations for steady state occupation numbers S_n we readily obtained the explicit expression for the total current

$$i_{Ca+Na} = -e_0 \frac{2(k_1^{Ca} - k_{-1}^{Ca} x_{Na}/y_{Ca})[Ca]/K_{Ca} + (k_1^{Na} - k_{-1}^{Na} x_{Na}/y_{Na})}{1 + [Ca]/K_{Ca}(1 + x_{Ca}/y_{Ca}) + x_{Na}/y_{Na}} \tag{1}$$

where $K_{Ca} = b/a$ is the dissociation constant of Ca ions with a high affinity regulatory site in the calcium channel.

The corresponding current voltage curves are shown in Fig. 2. When both Na and Ca ions are present in extracellular solution only, the theoretical curves reproduce well the effect of the depression of the sodium current through the calcium channel in the micromolar range of external Ca concentrations and the appearence of the calcium inward current at millimolar Ca concentrations.

The behavior of I—V curves with Na ions present in the intracellular solution only was somewhat unexpected. Upon lowering the extracellular Ca concentration the inward calcium current decreases whereas the outward sodium current increases. Superposition of these currents gives I—V curves with a clear reversal potential. The same effect was observed for calcium channels in chromaffine cells (Fenwick et al. 1982), isolated cardiomyocytes (Lee and Tsien 1984) and myeloma cells (Fukushima and Hagiwara 1985) when the calcium conductance could be effectively isolated from other ionic currents. Unfortunately, we could not observe this effect in neuronal membranes because their potassium conductance is difficult to eliminate using known pharmacological agents.

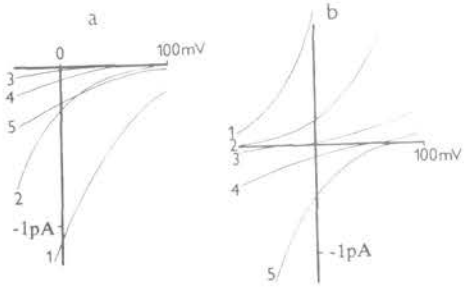

Fig. 2. I—V curves for the calcium channel with different conformational states calculated according to Eq. (1) at various extracellular Ca concentrations. The concentration of monovalent (Na) ions in extracellular (A) or intracellular (B) solutions was 30 mmol/l. The concentration of Ca ions was 2 μmol/l (1), 20 μmol/l (2), 0.2 (3), 2 (4) 20 (5) mmol/l. The height of the potential barriers for Ca and Na ions was 10 RT, the depths of the potential wells were -5 and 0 RT, corresponding to dissociation constants $K_{Ca} = 5$ mmol/l and $K_{Na} = 1$ mol/l, respectively (Kostyuk et al. 1982). The dissociation constant of Ca ions with the high affinity Ca binding site of the channel was 0.2 μmol/l (Kostyuk et al. 1983).

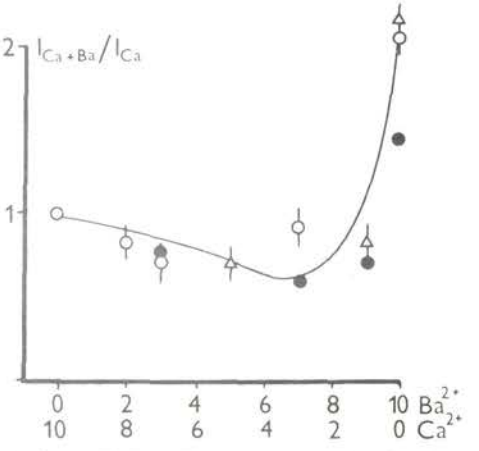

Fig. 3. Prediction of anomalous mole fraction behavior of mixed currents of alkaline earth metal cations by the model of the calcium channel with different conformational states. The curve was drawn using currents calculated according to Eq. (2) at zero membrane potential. The height of the potential barriers for Ca and Ba ions was 10 RT, the depths of the potential wells inside the channel were -5 and -3 RT, and the dissociation constants for high-affinity Ca binding site were 0.2 and 20 μmol/l for Ca and Ba ions, respectively. Points represent experimental data obtained by Hess and Tsien (1984) (●), Almers and McCleskey (1984) (△), Byerly et al. (1985) (○).

For these calculations we used the experimental dissociation constants and a minimum number of free parameters, assuming that the channel energy profile in each conformational state could be represented by a simple one-site model with equal and symmetrically shaped barriers. Our experience indicates that a quantitative description of I—V curves measured in the above works, can be obtained, e.g. by making the Na energy profile slightly asymmetrical.

For two penetrating alkaline earth cations (e.g. Ca and Ba), the kinetic scheme of the calcium channel with different conformational states is

$$S'_{Ba} \underset{y_{Ba}}{\overset{x_{Ba}}{\rightleftarrows}} S_{Ba} \underset{b_{Ba}}{\overset{a_{Ba}}{\rightleftarrows}} S_o \underset{b_{Ca}}{\overset{a_{Ca}}{\rightleftarrows}} S_{Ca} \underset{y_{Ca}}{\overset{x_{Ca}}{\rightleftarrows}} S'_{Ca}$$

where S_{Ba} and S'_{Ba} are the empty and Ba-conducting states of the channel. The expression of the total current is

$$i_{Ca+Ba} = -2e_0 \frac{(k_1^{Ca} - k_1^{Ca} x_{Ca}/y_{Ca})[Ca]/K_{Ca} + (k_1^{Ba} - k_{-1}^{Ba} x_{Ba}/y_{Ba})[Ba]/K_{Ba}}{1 + (1 + x_{Ca}/y_{Ca})[Ca]/K_{Ca} + (1 x_{Ba}/y_{Ba})[Ba]/K_{Ba}} \tag{2}$$

Fig. 3 shows the dependence of mixed Ca + Ba current through the calcium channel on extracellular Ba^{2+} keeping the total concentration of divalent ions constant. This theoretical curve reproduces well the anomalous mole fraction behaviour observed for calcium channels in different preparations.

Thus, we have shown that various anomalous mole fraction effects observed recently in Na/Ca and Ca/Ba mixtures can be succesfully described on postulating different conformational states of the channel. The binding of Ca^{2+}, Ba^{2+} (and possibly Na^+) to high-affinity sites may switch the transition of the channel to different conducting states. These conformational transitions are an intrinsic property of Ca-binding proteins. As to the calcium channel, they may participate in its gating mechanisms. In this respect, studies of possible relationships between the gating and the ion-transporting machinery of the calcium channel should be mentioned. Recently, it was shown for calcium channels fromrat brain (Nelson et al. 1984) and *Aplysia* neurones (Chesnoy-Marchais 1985) that the channel conductance and its mean open time are inversely related. That is, the stronger a given ion binds to the channel the lower is its condutance and the larger is its open time. Obviously, even this relationship should be expected from the model of a channel with different conformational states.

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