conditions of low $[Ca^{2+}]_i$, pCa > 7.0, the CBP gate (site 2) is open and the channel can be opened in a voltage-dependent manner. As $[Ca^{2+}]_i$ rises (pCa < 7.0), $Ca^{2+}$ binds to this CBP (site 2), closing or inactivating the channel (Fig. 2 *A*).

Using CDR as a model for the CBP gate of the channel, we recall that protein or drug binding to CDR is able to increase its affinity for $Ca^{2+}$ by nearly tenfold (10). Our unpublished results show that R24571 binding to CDR can increase the $Ca^{2+}$ affinity of the $Ca^{2+}$ sites that regulate felodipine binding to CDR by as much as 50-fold.

If the CBP of site 2 is similar to CDR in this regard also, then its binding of Ca-ANT could increase its affinity for $Ca^{2+}$ enough to bind $Ca^{2+}$, close the channel, and keep it closed even under low $[Ca^{2+}]_i$ conditions (Fig. 3 *B*). Thus, Ca-ANT may bind to the CBP gate of the channel, potentiate $Ca^{2+}$ binding to this protein, and thereby facilitate the action of $[Ca^{2+}]_i$ in closing or inactivating the channel. Under these circumstances, $Ca^{2+}$ channel blockers are perhaps more $Ca^{2+}$- agonists than $Ca^{2+}$-antagonists, since they facilitate the action of intracellular $Ca^{2+}$ with respect to channel blockade. Ca-ANT could vary in the affinity and/or $Ca^{2+}$ dependence of their binding to this CBP gate and in their ability to increase its affinity for $Ca^{2+}$, which might explain the differences in effectiveness and frequency-dependence of various Ca-ANT's. Different Ca-ANT may have different allosterically-related binding sites on this CBP, explaining the ability of some drugs to potentiate the binding and action of other drugs.

Consistent with our hypothesis, we note that not all $Ca^{2+}$ channels are inactivated by high $[Ca^{2+}]_i$. We would predict that these channels do not have a CBP gate and would, therefore, be insensitive to Ca-ANT drugs. It is well documented, for example, that the $Ca^{2+}$ channels regulating excitation-secretion are unique in that they are not inactivated by high $[Ca^{2+}]_i$ and are not sensitive to Ca-ANT drugs. This could reflect their lack of a CBP that gates the channel and binds $Ca^{2+}$ channel blockers.

This hypothesis is particularly attractive because at least two of its predictions are easily testable using current methodologies. (*a*). We would predict that Ca-ANT cannot block $Ca^{2+}$ currents in the presence of high concentrations of intracellular $Ca^{2+}$ chelators (EGTA, EDTA). (*b*) Ca-ANT binding to sarcolemma membrane preparations rich in $Ca^{2+}$ channels should increase their affinity for $Ca^{2+}$ in the same drug-dependent fashion as these drugs block $Ca^{2+}$ influx. We submit this concept as a testable hypothesis for further study.

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# PROPERTIES OF A CATION CHANNEL OF LARGE UNIT CONDUCTANCE IN LYMPHOCYTES, MACROPHAGES AND CULTURED MUSCLE CELLS

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Using the patch-clamp method (1, 2), we have identified a cation channel with novel properties in normal and transformed nonexcitable cells as well as in excitable cells. For experiments with nonexcitable cells, cultured FO-cells, thymocytes, and peritonal macrophages of mouse were used.



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## MATERIAL AND METHODS

The culture procedure for FO-cells was similar to that already described (3). FO-cells are a special type of myeloma cells that neither produce nor secrete immonoglobulins, and are hypoxanthin-phosphoryl-guanosin-transferase (HPGRT)-negative. Suspensions of thymocytes were isolated from the thymus of *Balb-c* mouse by conventional methods (4). For the isolation of peritonal macrophages the following procedure was performed. The *Balb-c* mouse was sacrificed and through a small hole in the abdominal wall 3 ml of RPMI 1640 (Gibco Diagnostics, Chagrin Falls, OH) were filled in. The abdomen was massaged on a vibrator and the 3 ml of medium were then sucked out. Cells were washed once through 30 ml of RPMI 1640 and the resuspended cells were incubated 1 h at 37°C.

Cultured chicken myotubes were obtained from 11-day old chicken embryo myoblasts which were fused after 50 h in culture by increasing the Ca-concentration of the culture medium to 1.4 mM (5).

The electrolyte within the patch-pipette as well as in the extracellular medium contained for all experiments (unless stated otherwise) NaCl, 137 mM; KCl, 5.4 mM; $CaCl_2$, 1.4 mM; HEPES, 10 mM; glucose, 10 mM; pH 7.4 at 20°C–22°C.

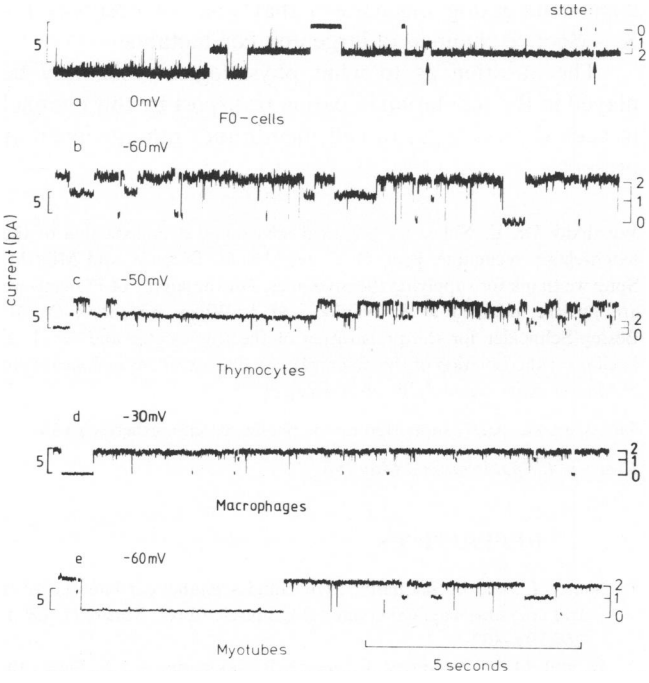


FIGURE 1 Single-channel currents from membrane patches of different cell types and different potential of the patch electrode. On the right ordinate the different current states of a channel are denoted: O-state is the ground state; 1 state is the substate or partially open state; and 2 state is the fully open state. *a*, current record obtained spontaneously from an intact FO-cell. For compostion of bath and pipette solution see Materials and Methods. In two cases (see arrows) the substate 1 is clearly visible due to its longer life time. *b*, current record of an inside-out patch of a FO-cell. The pipette fluid was as used for the record of Fig. 1 *a*, but after formation of the inside-out patch the bath medium was replaced by: KCl, 144 mM; 0.5 $\mu$M $Ca^{2+}$ (10 $\mu$M $CaCl_2$, 14 $\mu$M EGTA); HEPES, 10; pH, 7.4. The potential in the pipette was clamped to −60 mV. *c*, current record from a membrane patch of an intact thymocyte. Electrolyte solutions were as cited under Fig. 1 *a*. The pipette potential was set to −50 mV. *d*, current record obtained from an inside-out patch of a macrophage membrane. The solutions were as used for Fig. 1 *a*. The pipette potential was set to −30 mV. *e*, current record from an inside-out patch of myotube membrane. Electrolyte solutions were as used for Fig. 1 *a*. The pipette interior was set to −60 mV.

## RESULTS

From a membrane patch of an intact FO-cell in a few cases (four out of 53 gigaseals) current pattern as shown in Fig. 1 *a* could be recorded spontaneously with heat-polished micropipettes. Isolation of the active patch yielding to an inside-out patch caused no obvious change in the current pattern (compare Fig. 1 *a* and *b*) even for a Ca-free electrolyte ($10^{-7}$–$10^{-8}$ M $Ca_e^{2+}$). In ~30% of all cases an excise of a silent patch after a time lag of 3–5 min caused the appearance of a current pattern as shown in Fig. 1 *b*. This was independent of $Ca_e^{2+}$. Within the current fluctuations three distinct current states could be identified. We may denote these states as state O (closed state), state 1 (substate) and state 2 (fully open state) (see Fig. 1). The opening and closing kinetics of this channel appear to be rather complex and will be described in detail in a later report.

The most obvious observations are that the current preferentially fluctuates between state 2 and the closed state, omitting state 1, and that transitions of the sequence O⟶1⟶O were not observed within the time-resolution of ~500 $\mu$m of our setup. In Fig. 2 the current-voltage relationship of substate 1 and state 2 are plotted as measured with a cell-attached micropipette. The linear slopes in Fig. 2 indicate an ohmic behavior of the corresponding channel conductances (see also Table I). The reversal potential $V_r$ for state 1 and state 2 (Table I) was found to be identical in all experiments, but the absolute value varies from cell to cell. This may be caused by variation of the cell-membrane potential, $V_m$. The channel could be inactivated by increasing the membrane patch potential above a certain threshold in either a hyperpolarizing or a depolarizing direction. This voltage-dependent inactivation is reversible and shows little variation for the

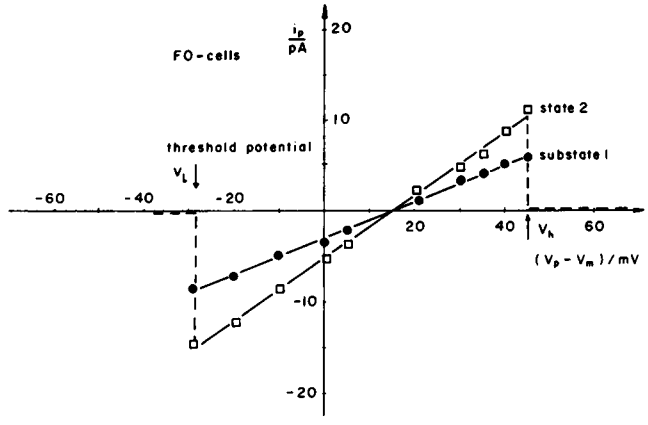


FIGURE 2 Single-channel current amplitude ($i_p$) as a function of applied potential ($V_m - V_p = V$) at the membrane patch referred to the cytoplasmic side. $V_p$ denotes the total membrane potential at the patch. The data were obtained spontaneously from a patch measurement on an intact FO-cell. □, single-channel current amplitude of state 2; •, single-channel current amplitude of substate 1. Outside certain threshold potentials $V_l$ and $V_h$ (marked by arrows) the channel closes permanently.

TABLE I
SINGLE-CHANNEL PARAMETERS

| Cell-type | Conductance $\gamma_{02}$/pS | Conductance $\gamma_{01}$/pS | $r$ | Reversal potential $(V_r - V_m)$/mV | $n$ |
|---|---|---|---|---|---|
| FO-cell | 335 ± 19 | 210 ± 18 | 0.63 | 20 ± 8 | 8 |
| Thymocyte | 234 ± 32 | 135 ± 28 | 0.58 | 17 ± 10 | 3 |
| Macrophage | 321 ± 24 | 195 ± 21 | 0.61 | 21 ± 6 | 11 |
| Myotube | 342 ± 21 | 201 ± 14 | 0.59 | 43 ± 8 | 6 |

$\gamma_{02}$ denotes the conductance of the fully open channel and $\gamma_{01}$ the conductance difference between state 0 and state 1. $r$ gives the ratio $\gamma_{01}/\gamma_{02}$. $V_r$ denotes the reversal potential measured under cell attached conditions, $V_m$ the cell membrane potential (not measured). $n$ is the number of independent experiments. The bath and pipette solution were identical and as described in Material and Methods. Values are given as mean ± SD of mean.

threshold potential of ~±3 mV within a single experiment. Isolation of the active patch shifts $V_r$, as expected, to ~0 mV for symmetrical solutions on both sides of the patch. Substitution of nine-tenths of the $Na^+$ in the bath solution by choline caused a shift of $V_r$ by 51 mV, indicating a cation permeability of the channel. No significant conductance change was observed on isolated membrane patches when KCl or CsCl was substituted for NaCl either on the cytoplasmic side of the membrane or within the pipette, indicating a lack of cation selectivity.

The single-channel properties as derived from membranes of transformed lymphocytes were compared with those obtained from membranes of normal lymphocytes. For this study, freshly prepared suspensions of thymocytes were used. For lack of contact between cell membrane and the bottom of the culture dish only one out of ~18 attempts yielded a tight seal. Under cell-attached conditions we again could observe current fluctuations (Fig. 1 *c*) that are strikingly similar to those of FO-cells (Fig. 1 *a*, *b*). From the corresponding current-voltage relationship the conductance of state 1 and state 2 ($\gamma_{01}$ and $\gamma_{02}$) as well as $V_r$ could be derived (Table I). We failed in isolating the membrane for an inside-out patch.

Under cell-attached conditions on macrophage membranes, current patterns as shown were observed in <10% of all experiments, whereas the excise of the membrane patch evoked current fluctuations as shown in Fig. 1 *d* in ~80% of all cases. This finding was independent of $Ca_e^{2+}$ in the range $10^{-8}$ to $10^{-3}$ M. The values of $\gamma_{01}$, $\gamma_{02}$ and $V_r$ are summarized in Table I.

For experiments with excitable cells we used cultured chicken myotubes. In rare cases (one out of 25 seals) current fluctuations of the shown pattern were observed spontaneously on a membrane patch of an intact myotube. In Fig. 1 *e* the corresponding current pattern obtained under excised patch conditions is shown. For isolated patches the kinetic pattern was obviously not affected by varying $Ca_e^{2+}$ in the range $10^{-8}$ to $1.4 \cdot 10^{-3}$ M. For the common reversal potential of state 1 and state 2 we obtained $V_r \approx V_m + (43 \pm 8)$ mV, varying from preparation to preparation. Assuming, for the myotubes used, a resting potential of ~ −50 mV, $V_r$ supports the finding that the channel is unselective for $Na^+$ and $K^+$.

Despite the very different ontogenetic origin of the cells used for this work, the kinetic pattern as well as the current amplitudes of this cation channel seem to be strikingly similar. Also, no obvious variation in the lack of ion selectivity or in the independence of channel activation by cytoplasmic $Ca^{2+}$ ($Ca_i^{2+}$) was observed for the different cell systems. In its kinetic pattern as well as the single channel conductance this channel is similar to the $Ca_i^{2+}$-dependent $K^+$-selective channel (6) at $Ca_i^{2+} \gtrsim 100\ \mu M$ and also to the $Ca_i^{2+}$-independent $K^+$-selective channel (7). However, from the data presented here the described channel is cation-unselective and shows a strong voltage-dependent gating mechanism that was not observed for $K^+$-selective channels of large unit conductance.

The question as to what physiological role may be played in the regulation of cation transport by this channel in such diverse types of cell membranes remains open at present.

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