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A class of non-selective cation channels in human fibroblasts

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Non-selective cation channels were detected in membrane patches of cultured human fibroblasts. The channels had a unitary conductance which ranged from 14 to 25 pS in symmetrical 130 mM NaCl and were permeable to both sodium and potassium ions. Open channel probability was dependent either on the membrane potential and the Ca^{2+} concentration on the intracellular side of the membrane. High Ca^{2+} concentrations in the millimolar range were needed to keep the channel active.

Cation channel; Patch-clamp technique; (Human fibroblast)

1. INTRODUCTION

The electrical properties of the membrane of human fibroblasts have extensively been studied by means of patch-clamp technique [1], yielding to the characterization of many types of ion channels including potassium channels [2,3], chloride channels [4,5], and stretch-activated channels [6]. Little is known about the physiological role of all these channels, but they are probably involved in important cellular processes like signal transduction across the membrane, cell volume and membrane potential regulation. In particular, it is possible that Ca^{2+}-dependent K^+ channels contribute to the control of membrane potential. In a previous study, for example, oscillatory hyperpolarizations in mouse fibroblasts have been attributed to the Ca^{2+}-mediated activation of a K^+ current [7]. In the present paper, we present evidence that in fibroblasts the intracellular Ca^{2+} might regulate another type of ion channel: a class of non-selective cation channels which can play an important role in the control of cell resting potential.

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2. MATERIALS AND METHODS

Human skin fibroblasts were cultured as described elsewhere [4] and used for patch-clamp experiments [1] in both confluent and non-confluent conditions. In the latter one, patches were obtained either from mitotic or interphasic fibroblasts. The experiments were carried out at room temperature (20–22°C) and in cell-attached, outside-out, and inside-out configurations. All the solutions, whose composition is reported in table 1, were adjusted to pH 7.3 with NaOH or KOH and to 290 mosmol/kg with mannitol. At the beginning, the bath and the pipette contained a symmetrical 130 mM NaCl (A1) solution. After excision of the patch, the solutions were changed by means of a perfusion pipette [8], with a flux rate of around 1 ml/min. Control experiments indicate that the solution in the patch vicinity was completely changed within 1–2 s. For excised patches, the membrane potentials and single-channel currents are given by using the usual sign conventions. Data acquisition and analysis were performed as previously described [3].

3. RESULTS

In inside-out patches, single channels of small conductance were revealed. The conductances ranged from 14 to 25 pS, with a mean value of 19.3 ± 3.8 pS (n = 18). As apparent from the traces of fig.1, different conductance values were observed even in the same patch. At present, it is not possible to distinguish if such different conductances represent separate types of channels, or

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Table 1

Composition of the solutions used in the experiments

	NaCl	KCl	$CaCl_2$	EGTA	Na-Hepes	K-Hepes
A1	130	–	2.00	–	10	–
A2	40	–	2.00	–	10	–
B1	–	130	2.00	–	–	10
B2	–	130	0.20	–	–	10
B3	–	130	1.98	2	–	10
B4	–	130	1.80	2	–	10

The concentrations are given in mM. The free Ca^{2+} concentrations for the (B3) and (B4) solutions are 1×10^{-5} M and 1×10^{-6} M, respectively

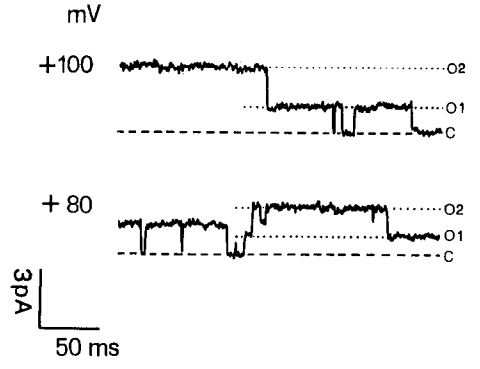

Fig.1. Single-channel currents recorded from an inside-out patch in symmetrical 130 NaCl (A1 solution). The traces, obtained at the indicated membrane potential, show openings of two channels (O1 and O2) with different current amplitudes. The baseline is represented by a dashed line whereas the open channel levels are indicated by dotted lines. The analysis of the single-channel current performed in the range of membrane potentials from −100 to +100 mV gives two conductance values of 15 and 21 pS for O1 and O2, respectively.

different conductance levels of the same type of channel. However, when a salt gradient was created, these channels showed a common selectivity for cations. In fact, when an inside-out patch from a symmetrical condition of 130 mM NaCl was exposed to a gradient of 130 mM NaCl outside/40 mM NaCl inside (see fig.2), the zero-current potential was shifted in the positive direction from 0 to a value of 28.3 ± 1.6 mV (n = 7). This value is close to the theoretical 30 mV predicted from the Nernst equation for a channel purely selective for Na^+ over Cl^-. When in the same experiments, the patches were perfused with 130 mM KCl (B1) solution, the zero-current potential only shifted to 7.0 ± 1.5 mV (n = 8), which means that the channel is also permeable to K^+, with a slight preference for Na^+. According to the Goldman-Hodgkin-Katz equation, the permeability ratios P_{Cl}/P_{Na} and P_K/P_{Na} are 0.03 and 0.78, respectively.

Channel activity in inside-out patches was affected by changes of the membrane potential and of the Ca^{2+} concentration on the cytoplasmic side of the patch (see fig.3). In the presence of 2 mM Ca^{2+} in the bath solution, the open channel probability (P_o) increased in a sygmoidal fashion as the patch was made more positive. The voltage dependence corresponds to an e-fold change of the ratio $P_o/(1 - P_o)$ for a 50 mV change of membrane potential. Lowering the Ca^{2+} concentration to 0.2 mM, caused the activity to be reduced, although the activity was still voltage dependent. Instead, no channel openings were detected with free Ca^{2+} concentrations lower than 0.2 mM (B3, B4 solutions). After restoration of the high Ca^{2+} on the internal side of the membrane, the channel activity recovered almost immediately.

Cation channels were also recorded in the cell-attached configuration (see fig.4). In these conditions channel openings, with conductance around 20 pS, were also observed at voltages close to the cell resting potential (V_{pip} = 0). This means that the channel can be normally active in physiological conditions.

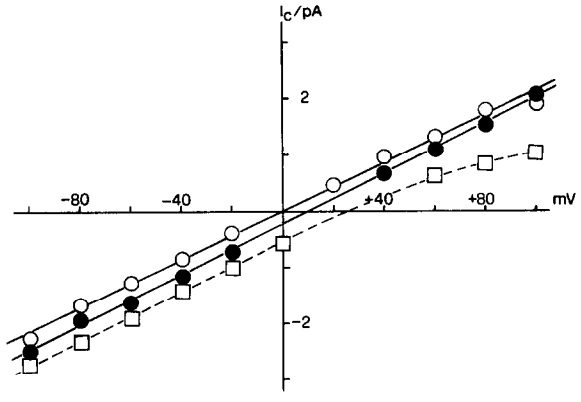

Fig.2. Current-voltage relationships for a cation channel in an inside-out patch. The bath and the pipette initially contained 130 mM NaCl symmetrical (A1) solution (○). The bath solution was subsequently replaced with 40 mM NaCl (A2) solution (□) and 130 mM KCl (B1) solution (●).

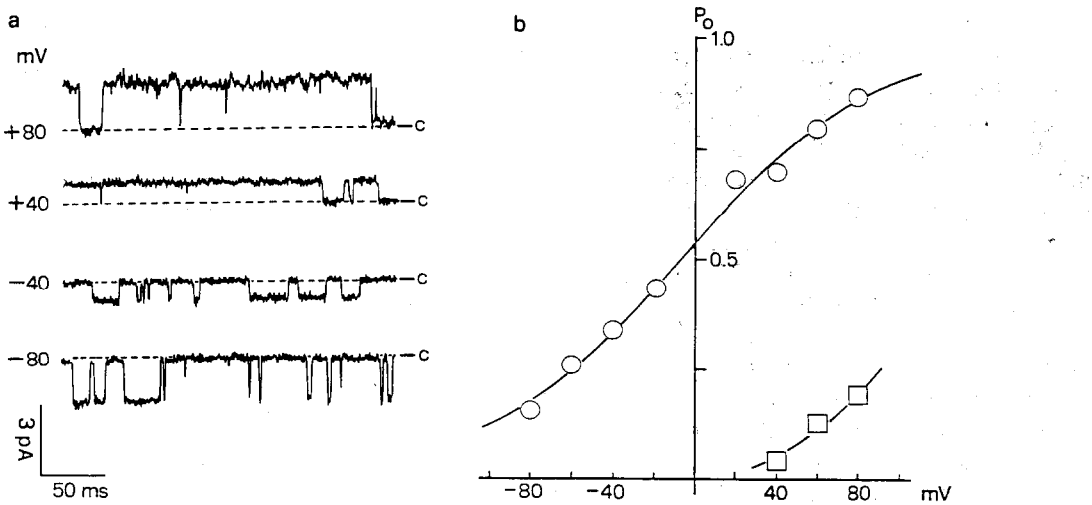

Fig.3. (a) Single-channel currents, recorded from an inside-out patch at different membrane potentials. The closed channel level is indicated by the dashed line. The traces were obtained with 2 mM Ca^{2+} in the bath (B1 solution). (b) Plot of the open channel probability (P_o) versus the membrane potential obtained at the same Ca^{2+} concentration (B1 solution) (○). Experimental points were fitted with the Boltzmann equation. The decrease of the Ca^{2+} concentration to 0.2 mM (B2 solution) (□) was sufficient to drastically reduce the channel activity by shifting the P_o-V_m curve in the positive direction.

A typical K^+ channel blocker, quinine, was tested in excised patch configurations. This blocker, at the concentration of 1 mM, was effective in producing a reversible total block of channel currents from either sides of the membrane (data not shown).

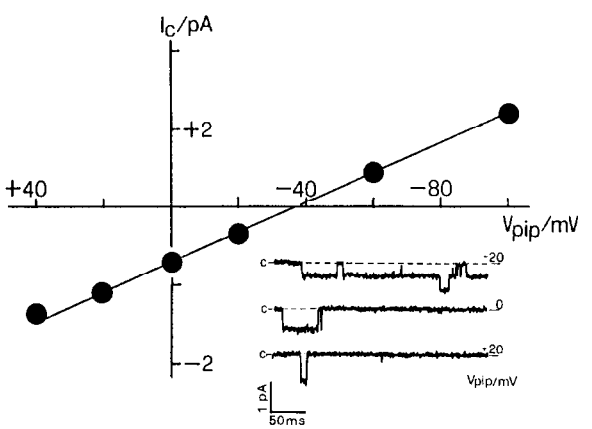

Fig.4. Current-voltage relationship and representative single-channel currents obtained from a cell-attached patch. The pipette and the bath contained the A1 solution. The mean values of single-channel currents are plotted versus the pipette voltage (V_{pip}) which represents the deviation from the resting potential. Positive and negative pipette voltages respectively hyperpolarize and depolarize the patch. Positive currents mean currents flowing from the cytoplasmic to the external side of the membrane.

4. DISCUSSION

The channels described in this paper are characterized by conductance values around 15–25 pS, strong selectivity for cations over chloride, a poor discrimination among cations, and Ca^{2+}-dependent activity. These properties are similar to those reported for Ca^{2+}-activated non-selective channels (CAN channels) which have been found in several types of cells (see [9] for review). Instead, a difference exists with respect to the voltage dependence because CAN channels in other preparations are virtually not voltage dependent [10–12], whereas in fibroblasts, as also reported for insulinoma [13] and Schwann cells [14], an appreciable sensitivity of channel activity to the membrane potential could be revealed. In all the experiments the open channel probability was dependent on the Ca^{2+} concentration facing the intracellular side of the patch, but the Ca^{2+} levels required to keep the channel active (2.0–0.2 mM) were very far from the physiological intracellular range. A similar low Ca^{2+}-sensitivity has been found in CAN channels of insulinoma [13] and Schwann cells [14] and also recently described for maxi-K^+ channels in human fibroblasts [3]. It is possible that the Ca^{2+} sensitivity of CAN channels is modulated by cytoplasmic factors which are lost

after patch excision. These factors could be represented by soluble proteins such as calmodulin or calmodulin-like proteins which may act directly or by means of protein kinases. Such a mechanism could explain why in fibroblasts we observed channel activity in cell-attached patches where the physiological internal Ca^{2+} levels should be actually much lower than those required for channel activation in excised patches. The results of this paper confirm the hypothesis formulated by means of ion flux measurements about the presence of non-selective cation channels in fibroblasts [15]. Further studies are needed to elucidate whether such channels are actually involved in the redistribution of Na^+ and K^+ which appears to be controlled by the extracellular Ca^{2+} and the protein kinase C [15].

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