Calcium-Permeable Channels in HeLa Cells

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Abstract. Calcium-permeable channels with a slope conductance of 9 pS were revealed in excised inside-out patches of cultured HeLa cells. Over a potential range from −80 to −10 mV, unitary inward currents were recorded with 110 Ca^{2+} in the pipette in artificial "intracellular" solutions containing impermeant anions. Channel activity was not considerably affected by varying free calcium concentration between 0.01 and 10 μmol/l in cytosol-like solution. In experiments with low spontaneous activity of calcium-permeable channels in the inside-out patch, it could be increased by the application of 5–10 μmol/l inositoltrisphosphate to the inner membrane surface.

Key words: Patch clamp — Carcinoma HeLa cell — Calcium-permeable channels — Inositoltrisphosphate

Introduction

Transient increases of cytosolic free calcium concentration in response to binding of hormones or other stimulant molecules with cell surface play an important role in transmembrane signalling (Berridge and Irvin 1989). It is well established that extracellular calcium influx through plasma membrane occurs together with its release from intracellular stores (Hallam and Rink 1989). Currently, the mechanisms underlying calcium entry in non-excitable cells are intensively studied. Patch-clamp studies confirmed directly the existence of calcium-permeable receptor-operated channels, principally postulated beforehand, in several cell membrane types: lymphocytes (von Tscharner et al. 1986; Kuno and Gardner 1987), A-431 carcinoma cells (Mozhayeva et al. 1989; 1991) sarcoplasmic reticulum vesicles (Ehrlich and Watras 1988). In human carcinoma HeLa cells stimulation of H1-receptors by histamine was shown to lead to biphasic rise of cytosolic free calcium concentrations partially mediated by inositoltrisphosphate (Tilly et al. 1990; Sauve et al. 1991). The contribution of the receptor-evoked Ca^{2+} influx was investigated by measuring the activity of calcium-dependent potassium channels and using fluorescent probe Fura-2 (Sauve et al. 1990). These data have allowed us to suggest that the plasma

membrane of HeLa cells contains some receptor-dependent channels permeable to calcium. Calcium signal generation due to some other transporters in cell membrane could not be excluded. The involvement of Ca^{+}-H^{+} exchange system in receptor-evoked calcium influx has also been supposed (Sauve et al. 1991). In the present paper we describe Ca^{2+}-permeable channels of rather high conductance; they may be a part of calcium entry mechanism in the plasma membrane of human carcinoma HeLa cells.

Materials and Methods

Experiments were performed on cultured HeLa cells. The cell culture was obtained from the Cell Culture Collection (Institute of Cytology, Russia). The culture was maintained in glass flasks in Dulbecco modified Eagle medium (DMEM, Gibco), supplemented with 10% bovine serum inactivated at 56 °C. Two to four days before experiment, the cells were transferred into plastic Petri dishes and plated on coverslips (0.4 × 0.4 cm). For single current measurements the coverslips were placed into a working chamber filled with solution containing (mmol/l): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES-TrisOH, 10 glucose. Single-channel currents were recorded using the inside-out recording configuration with a List-EPC7 patch-clamp amplifier. Current signals were stored on analogue tape and digitized for analysis.

Hard glass pipettes were filled with solution containing (mmol/l) 100 $CaCl_2$ and 10 $Ca(OH)_2$/HEPES. After patch excising the pipette was moved to the small volume compartment (glass tube of the volume 0.01 ml) of the chamber. The cytosol-like "intracellular" solutions contained (mmol/l): 70 K_2SO_4 (or 140 K-arabonate), 20 HEPES/KOH (pH 7.3), 1 $MgCl_2$, 1 EGTA/KOH or 2 HEEDTA/KOH and appropriate quantity of $CaCl_2$ to establish a final free calcium concentration of 0.01–10 μmol/l. Inositoltrisphosphate (IP_3), HEPES, EGTA, HEEDTA were from Sigma. Experiments were carried out at 31–33 °C.

The probability for the channel being open (P_0) was calculated from the formula: ($P_0 = I/(N \cdot i)$, where I is the mean channel current; N is the number of functional channels in patch; i is the unitary current amplitude. Mean current I was determined from amplitude histograms obtained for 5–10 second time intervals. Mean values and S.E. are given.

Results

Figure 1*A*. shows a representative example of inward single-channel currents recorded from an inside-out patch excised from a HeLa cell. The membrane patch cytoplasmic surface was bathed in a solution containing 70 mmol/l K_2SO_4 with 0.01 μmol/l free Ca^2 concentration. The only cation in pipette solution was calcium. In these conditions inward currents can be carried only by calcium influx. Sulphate-ions are completely impermeable in patches excised from HeLa cells as shown in our previous work concerning anion channels (Savokhina et al. 1991). Furthermore, current amplitudes were the same whether sulphate or arabonate

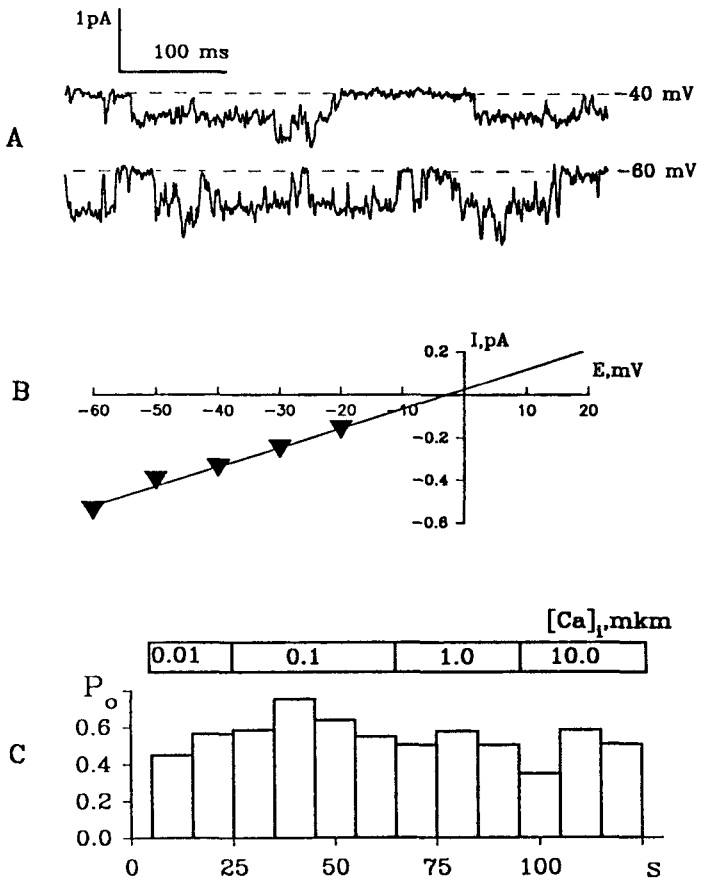

Figure 1. Channel activity in an inside-out patch of a HeLa cell. Cytosol-like solution contained 70 mmol/l sulphate as a main anion. Pipette solution contained (mmol/l) 100 $CaCl_2$ plus 10 $Ca(OH)_2$/HEPES mixture. *A*. Representative single current records in cytosol-like solution containing 0.1 μmol/l free Ca^{2+}. Holding membrane potentials are indicated at the traces. *B*. Unitary current-voltage relation. Single-channel conductance is 9.6 pS. *C*. Open probability in time corresponding to different free calcium concentrations in the cytosol-like solution. Membrane potential −40 mV.

was the major anion in the bath solution. There were at least two similar channels in this patch characterized by a unitary conductance of 9.6 pS as obtained from the current-voltage relation (Fig. 1*B*.). The linearly extrapolated current-voltage relation intercepts x-axis at approximately zero. Channels with similar conductive properties (9.4 ± 0.11 pS, linearly extrapolated reversal potential $+2.6 \pm 0.57$ mV) were observed in 7 inside-out experiments, and they appear to belong to the same

type of calcium-permeable channels.

The level of spontaneous activity in the experiment illustrated in Fig. 1 was rather high and, as can be seen from Fig. 1*C*., was not changed significantly by varying inner Ca^{2+} in a range from 0.01 to 10 μmol/l. Similar insensibility to inner Ca^{2+} was observed in two other experiments (also when P_0 has grown after IP_3 application, see later); thus the activity of this channel type did not appear to be dependent on free cytoplasmic calcium.

In 4 experiments spontaneous channel activity was low and could be significantly increased after inositoltrisphosphate application to the intracellular membrane side of the excised patch. The results of a typical experiment are shown in Fig. 2. Fig. 2*A*. presents the time course of IP_3-induced activation in terms of P_0 calculated on the assumption that three channels existed in the patch. The number of simultaneous openings on current records (see Fig. 2*B*.) and the corresponding number of peaks in amplitude histograms (not shown) suggest that at least three functioning channels were present in the patch. Prior to IP_3 application, only rare openings were observed during 1.5 min (P_0 below 0.005). It can be seen (Fig.2*A*.) that the activity started growing at 30th second after addition of 5 μmol/l IP_3 to the cytosol-like solution (0.1 μmol/l Ca), and P_0 reached a maximal value of 0.38 (for 5 s interval) within 70 seconds. Fig. 2*B*. shows single current records corresponding to the periods after reaching maximal activity. The current-voltage relation yielded a unitary conductance of 8.5 pS and an extrapolated reversal potential of about +10 mV (Fig. 2*C*.).

In different experiments the latency of activation following IP_3 application varied from 2–3 to 50 s. Then, the rising channel activity lasted several minutes (till membrane instability reached a critical level, usually 5–10 min). P_0 values altered significantly throughout each experiment, minimal P_0 values were about 0.02–0.05, maximal values reached 0.4–0.6. At the same time channel activity and the corresponding P_0 seemed to be independent on changes in the holding potential (Fig. 2*A*.) or inner free Ca^{2+} (Fig. 1*C*.). Being initiated, channel activity could not be abolished by washout of IP_3-containing solution. It should be noted that in two experiments IP_3 failed to produce an increase of channel activity.

Generally in our series of experiments most stable excised patches (61%) were silent (no inward calcium currents) before and after application of IP_3 and some other possible intracellular messengers. Under our experimental conditions channel activity displaying inward calcium currents was observed in 37% of stable inside-out patches. It should be mentioned that, besides the type of 9 pS calcium-permeable channels described here, another pool(s) of channels characterized by a considerably smaller conductance (about 1.5–2 and 3.5 pS) and more positive extrapolated values of reversal potential were observed in our patch experiments on HeLa cells.

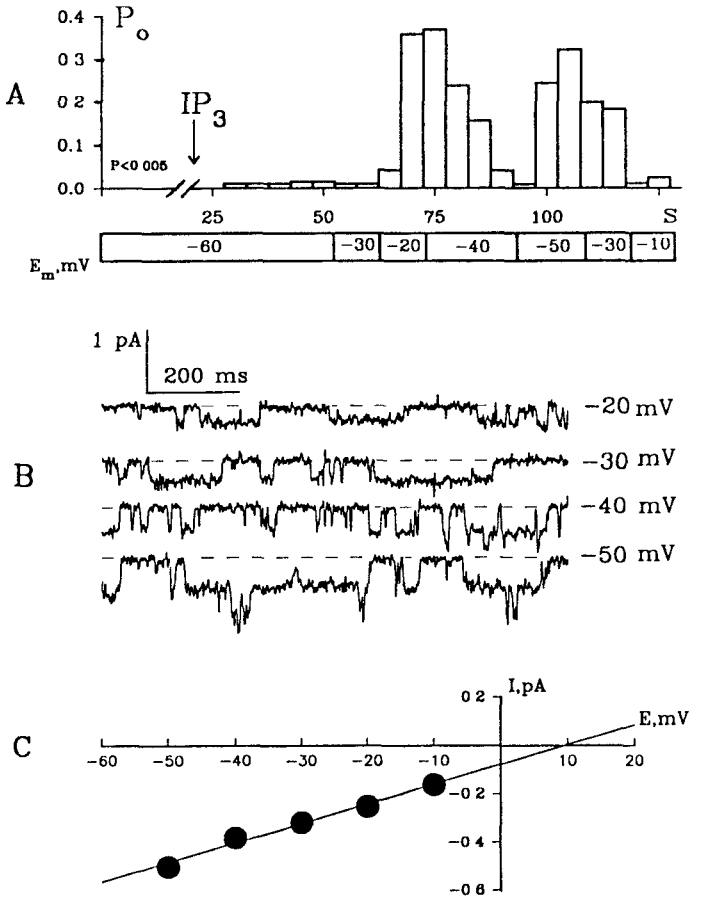

Figure 2. Activation of single channel currents in an excised patch after the application of 5 μmol/l inositoltrisphosphate to the intracellular membrane side. Free calcium concentration 0.1 μmol/l. *A*. Open probability in time. The arrow indicates IP_3 addition (the starting point of the time scale). *B*.Current records after IP_3 application. Membrane potentials are indicated at the traces. *C*. Unitary current-voltage relation. Single-channel conductance is 8.1 pS.

Discussion

The data obtained support the assumption that non-voltage-gated calcium-permeable cation channels exist in the plasma membrane of HeLa cells. Channels with similar Ca^{2+} conductance in excised patches were found in T-lymphocytes (Kuno and Gardner 1987) and in A431 human carcinoma cells (Mozhayeva et al. 1990) In

the latter case channels of 13 pS were reported to be also activated by inositol 1,4,5-trisphosphate applied to the intracellular side of the plasma membrane. Unlike the works mentioned, in our experiments the extrapolated values of the reversal potential proved to be close to zero. These channels could be suggested to have low cation selectivity and rather high permeability for potassium and other monovalent cations. Calcium-permeable channels with low cation selectivity were reported in BALB/c 3T3 fibroblasts (Matsunaga et al. 1988) but with two times greater conductivity (19 pS with 110 mmol/l Ba^{2+} in pipette solution and similar currents when isotonic $CaCl_2$ or NaCl, KCl was employed).

It may be assumed that cation channels found in our experiments participate in mediating Ca influx triggered by histamine (Sauve et al. 1990; Tilly et al. 1990) and consequently in forming Ca^{2+} signal in HeLa carcinoma cells. However, as can be followed from an estimate of their selective properties, under physiological conditions these channels appear to pass sodium ions together with calcium from the external solution. To evaluate the real contribution of ionic fluxes mediated by this and other channel types in physiological processes of signal transduction single-channel measurements in outside-out mode are in progress.

As for the possible role of IP_3 as the second messenger controlling cation entry in HeLa cells (Tilly et al. 1990) it seems likely that the substance acts as a switch turning on following activity of the calcium-permeable channels in the plasma membrane. The activation of channels like these, characterized by a relatively high conductance and long periods of active functioning, could provide the second part of a calcium signal in HeLa cells lasting at least several minutes (Sauve et al. 1990). It is the sustained calcium influx which has been shown to be evoked only in interphase but not in mitotic HeLa cells (Volpi and Berlin 1988). As an asynchronous cell culture was used in our experiments, the failure to activate calcium channels by IP_3 in some experiments may be also explained by the fact that cells were in different phases of cell cycle.

The mechanisms of the channel inactivation remains unclear. We observed no significant decrease of P_0 both in presence and absence of IP_3; variation of the inner free calcium concentration also proved to be ineffective in regulating channel activity. The data presented allow us to conclude that IP_3 is the intracellular messenger modulating the activity of 9 pS calcium-permeable channels in HeLa cell plasma membrane. It is likely that other substances, most probably G-proteins, are also involved in channel regulation.

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