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Inositol 1,4,5-trisphosphate activates two types of Ca^{2+}-permeable channels in human carcinoma cells

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Ca^{2+}-permeable channels in human carcinoma A431 cells were studied using the patch clamp technique. We have found two types of Ca^{2+}-permeable channels which are activated by inositol 1,4,5-trisphosphate (IP_3) applied to the intracellular side of the plasma membrane. Unitary conductances of these channels are 3.7 and 13 pS (105 mM Ca^{2+} in recording pipette, 30–33°C). From the extracellular side of the membrane the channels are activated by EGF. It is assumed that extracellular agonists open both channel types by stimulating the release of IP_3 from the membrane.

Patch clamp; Carcinoma cell; Calcium channel; Epidermal growth factor; Inositol 1,4,5-trisphosphate

1. INTRODUCTION

Calcium-mobilizing agonists induce a rise of intracellular concentration of free Ca by means of discharge of intracellular Ca^{2+} stores and Ca^{2+} entry from the extracellular medium [1]. It is well established that the release of calcium from intracellular stores is mediated by IP_3 which open Ca-permeable channels in the membrane of endoplasmic reticulum [2,3]. A number of recent electrophysiological studies [4–7] suggest that the plasma membrane also contains IP_3-sensitive pathways for calcium. Here we describe two novel types of Ca-permeable, responsive to IP_3 channels in the plasma membrane of human carcinoma A431 cells.

2. MATERIALS AND METHODS

Experiments were performed on cultured human epidermoid carcinoma A431 cells (Cell Culture Collection, Institute of Cytology, USSR) either at 20–23°C or 30–33°C. The cells were grown as described earlier [8].

Recording pipettes were filled with solution containing 100 mM $CaCl_2$ plus 5 mM $Ca(OH)_2$/Hepes mixture (pH 7.4). The bath solution for cell-attached experiments contained (in mM): 145 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 10 Tris-HCl (pH 7.4). The cytosol-like solution for inside-out experiments contained (in mM): 140 KCl (or K-aspartate, K-glutamate), 10 Hepes/KOH (pH 7.3), 5 EGTA/KOH, 1.5 $CaCl_2$, (pCa 7.5), 1 $MgCl_2$. IP_3 was obtained from Calbiochem. EGF from mouse submaxillary glands was a gift of Dr A.D. Sorkin.

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; EGTA, ethylene glycol-bis β-amino-ethylether) *N,N,N′,N′*-tetraacetic acid; EGF, epidermal growth factor

Single-channel currents were recorded using cell-attached and inside-out recording configurations [9]. Current signals were stored on analogue tape and digitized for analysis. The probability for each channel to be open (P_o) was calculated from the formula $P_o = <I>(N_f \times i)$, where $<I>$ is the mean channel current, N_f the number of functional channels in the patch, and i the unitary current amplitude. $<I>$ was calculated as time integral of patch current above baseline over 1–5 s intervals. N_f was taken to be equal to the maximum number of simultaneous openings.

Averaged data are given as mean ± SE.

3. RESULTS AND DISCUSSION

Fig. 1 shows conductive properties and response to IP_3 of channels referred further to as BI-channels. Channel currents are inwardly directed; linearly extrapolated current/voltage relation intercepts x-axis at about +20 mV. Current amplitudes were the same whether chloride or glutamate was the major anion in the artificial intracellular solution. These facts strongly suggest that the currents are carried by Ca^{2+} ions going from the pipette solution rather than anions going from intracellular solution. Channels with similar conductive properties were observed in 21 inside-out and cell-attached experiments, they seem to belong to the same BI-type. Their mean conductances (in pS) were 10.3 ± 0.3 ($n=8$) at 21–23°C and 12.8 ± 0.4 ($n=13$) at 30–33°C.

It can be seen from Fig. 1 that IP_3 caused a large increase in the channel opening; maximum P_o value (over 2 s interval) came up to 0.48. The elevated channel activity lasted no less than 60 s in this experiment (later, measurements became impossible due to growing instability of the patch membrane). A qualitatively similar effect of IP_3 on BI-channels was observed in 9 inside-out experiments.

IP_3 can activate channels of more than 1 type (I) in the membrane of A431 cells. Their unitary conductance

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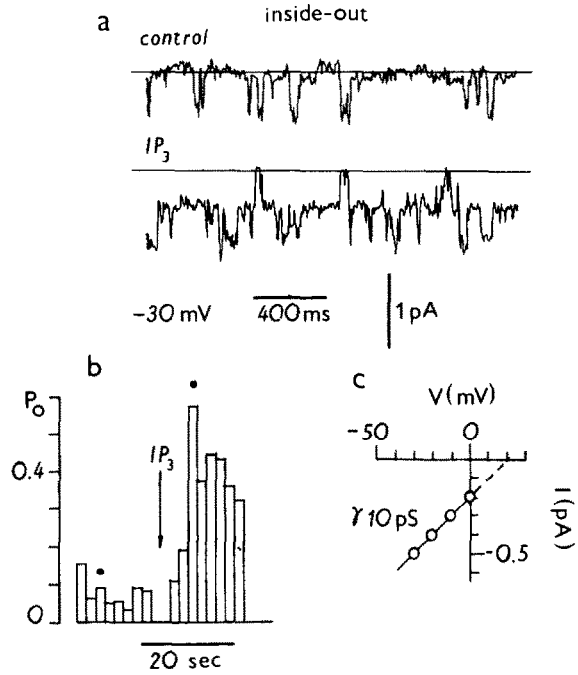

Fig. 1. BI-channel activity in inside-out patch. (a) Currents at −30 mV before and after the addition of 5 μM IP_3 to the intracellular solution. (b) The channel activity expressed in P_o. Shown are 16 s before and 16 s after the IP_3 addition. Filled circles indicate the time when the current records were taken. (c) Unitary current/voltage relation.

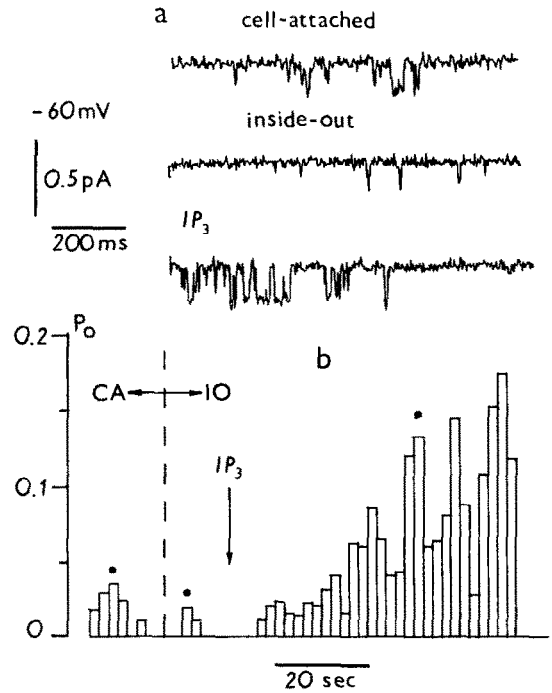

Fig. 2. Activity of I-channels in cell-attached (CA) and inside-out (IO) configurations in the same patch. EGF (8.3 nM) was added to pipette solution. (a) Currents at -60 mV. Unitary slope conductance was 3.5 pS in this experiment. (b) The channel activity before (CA) and after (IO) the patch excision. The addition of 5 μM IP_3 to the intracellular solution is marked by an arrow. Filled circles indicate the time when the current records were taken.

ranged from 3.1 to 3.7 (mean 3.31 pS) at 20–23°C and from 3.4 to 4.0 (mean 3.70 pS) at 30–33°C. Currents through I-channels were indifferent to whether chloride or organic anions were predominant in the intracellular solution, suggesting that a current-carrying ion is Ca^{2+}.

Stimulatory effect of IP_3 on the I-channel is shown in Fig. 2. In this experiment unfrequent channel openings were seen in cell-attached configuration, they disappeared after excision of the patch and reappeared on the addition of 5 μM IP_3 to the internal solution; the maximum value of P_o (for 2 s interval) was higher than 0.15. Similar, though less pronounced, elevation of the channel activity was observed in 5 analogous experiments in which IP_3 was applied alone. More intensive IP_3-induced activation of I-channels occurred in 9 other experiments in which non-hydrolyzable analogues of GTP (GTPγS) were applied prior to IP_3. In these experiments P_o values (for 2 s intervals) were up to 0.6. It should be noted that GTPγS by itself failed to produce a stimulating effect on I-channels. The effect of GTPγS on I-channels seems to imply that these channels are up-regulated by guanine nucleotide binding (G) proteins [10].

Cell-attached experiments demonstrated that BI- and I-channel activity could be also increased (4–7 times) by the addition of EGF (6.3–16.6 nM) either to bath or pipette solutions. In the latter case the activity was short-lived which seemed to be due to EGF being able to elevate IP_3 concentration [11] near intracellular surface of the patch membrane but for a short time because of a limited store of phosphatidylinositol 4,5-bisphosphate in it and IP_3 diffusion from the patch.

IP_3-dependent whole-cell Ca^{2+} currents were measured in mast cells [6] and T-lymphocytes [7]; in both cases the data suggested that these currents were mediated by channels with a low unitary conductance (less than 1 pS) at normal Ca^{2+} concentration (about 2 mM) in the bath solution. It is possible that IP_3-sensitive channels in T-lymphocytes and mast cells are analogous to I- or BI-channels.

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