Calcium-permeable channels activated via guanine nucleotide-dependent mechanism in human carcinoma cells

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Patch clamp experiments on human carcinoma A431 cells have revealed two types of Ca^{2+}-permeable channels, the activity of which can be increased by the application of non-hydrolyzable analogues of GTP to the intracellular side of the membrane. With 105 mM Ca^{2+} in recording pipette at 30–33°C their unitary conductances (in pS) are 1.3 (SG-channels) and 2.4 (G-channels). G- and, possibly, SG-channels are activated from the extracellular side of the membrane with epidermal growth factor (EGF). The data are consistent with the hypothesis that both channels are activated via guanine nucleotide binding (G) proteins.

Patch clamp; Carcinoma cell; Calcium channel; Guanine nucleotide; Epidermal growth factor

1. INTRODUCTION

The receptor-induced calcium influx plays an important role in transmembrane signalling in many cell types [1–4]. In nerve, muscle and other electrically excitable cells, Ca^{2+} influx is mediated by voltage-gated calcium channels. In non-excitable cells mechanisms of Ca^{2+} entry are less certain. The data obtained in tracer flux and Ca^{2+}-sensitive dye experiments [5,6] suggest that the plasma membrane of many cells contains Ca^{2+}-permeable channels different from long-known voltage-gated ones. The most direct approach to demonstrate the existence of these hypothetical channels and determine their functional properties is provided by patch clamp methods [7]. There are only few studies [8–10] reporting functional properties of some receptor-dependent, Ca^{2+}-permeable channels. In the present paper we give single-channel description of two novel Ca-permeable channels which seem to be directly gated by G proteins.

2. MATERIALS AND METHODS

Experiments with the use of cell-attached or inside-out versions of the patch clamp technique [7] were performed on human carcinoma A431 cells (Cell Culture Collection, Institute of Cytology, USSR).

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Abbreviations: EGF, epidermal growth factor; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; GppNHp, 5'-guanylyl-imidodiphosphate; GTPγS, 5'-*O*-(3-thiotriphosphate)

For these experiments the cells were plated on coverslips and grown in basal Eagle's medium supplemented with 10% bovine or fetal serum, 30 μg/ml gentamycin in 6% humidified atmosphere to half- or near-confluency.

The pipettes were filled with 100 mM $CaCl_2$ plus 5 mM $Ca(OH)_2$/Hepes mixture (pH 7.4). When necessary, EGF was added to this solution. When working in cell-attached configuration we used the potassium bath extracellular solution, containing (in mM) 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Tris-HCl (pH 7.4) to nullify the resting potential on the extrapatch membrane. The cytosol-like solutions contained (in mM) 145 KCl (or K-aspartate, glutamate), 10 Hepes/KOH (pH 7.3), 5 EGTA/KOH, 1.5 $CaCl_2$ ($Ca^{2+} \leqslant 10^{-7.5}$M), 1 $MgCl_2$, GTPγS and Hepes were from Sigma; GppNHp from Serva; EGTA, from Fluka. EGF from mouse submaxillary glands was a gift of Dr A.D. Sorkin.

Experiments were carried out at 20–23°C or at 30–33°C. Before digitization and inserting into a computer-stored current signal was filtered at frequencies from 0.5 to 0.1 kHz. The probability for the channel to be open (P_o) was calculated from the formula: $P_o = <I>/(N_f \times i)$, where $<I>$ is mean channel current; N, the number of functional channels in the patch; i the unitary current amplitude. $<I>$ was estimated from the time integral of patch current above baseline over 5 s interval. N_f was taken to be equal to the maximum number of simultaneous openings.

3. RESULTS AND DISCUSSION

Characteristic properties of the channels referred further to as to G-channels are shown in Fig. 1. In this experiment the current measurements were performed firstly in cell-attached and then in inside-out configurations. Current amplitudes were the same both in cell-attached and inside-out patches; in the latter case they remained unchanged on substitution of glutamate by chloride anions in the artificial intracellular solution. The current/voltage relation shows slope conductance of 2.5 pS and being extrapolated intercepts x-axis at about +30 mV. A positive value of reversal potential

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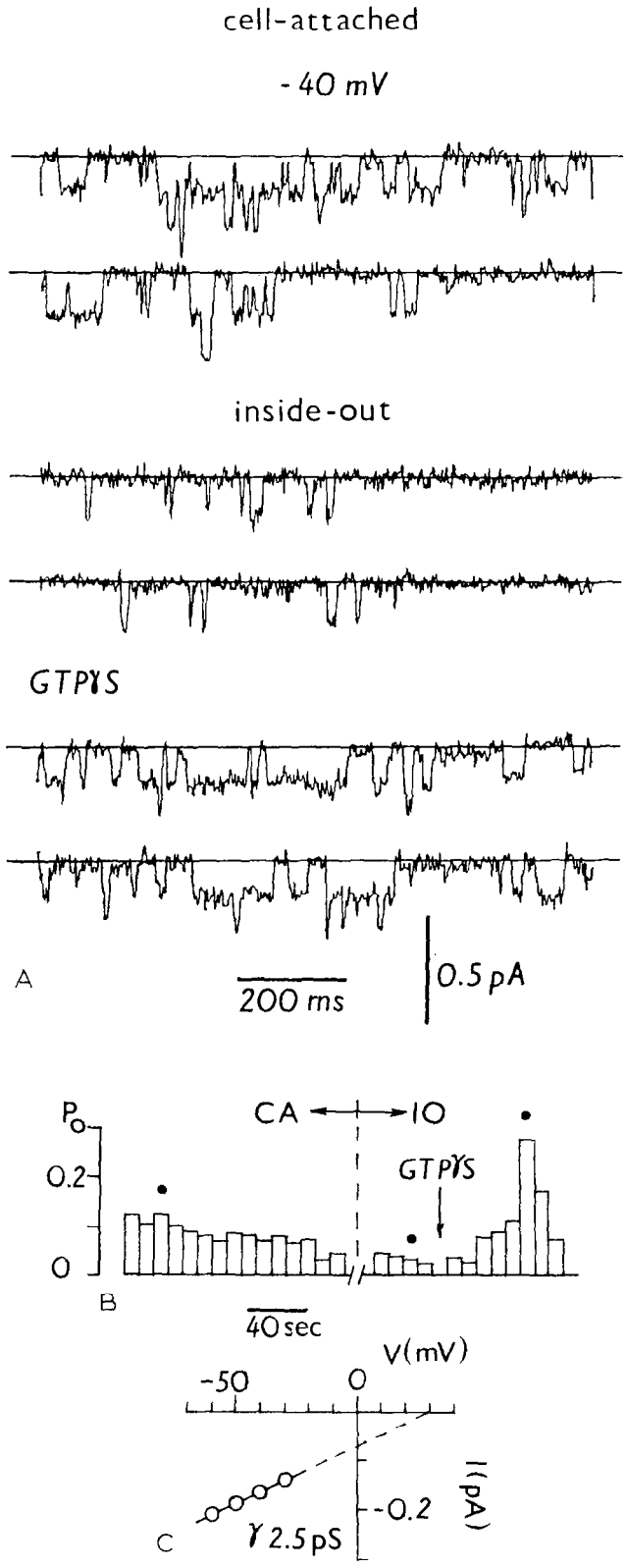

Fig. 1. Properties of G-channels. (A) Representative current records at −40 mV in cell-attached and inside-out configurations before and after the application of 100 μM GTPγS. (B) Channel open probability (P_o) in time before (CA) and after (IO) the patch excision. Filled circles indicate the time when current records were taken. (C) Unitary current/voltage relation.

and the insensitivity to intracellular anions suggest that inward currents through these channels are carried by Ca^{2+}, the only cation in the pipette, rather than by anions going from the intracellular solution.

In this experiment EGF (6.3 nM) was added to the pipette solution and apparently owing to this, the channel activity in the cell-attached configuration was

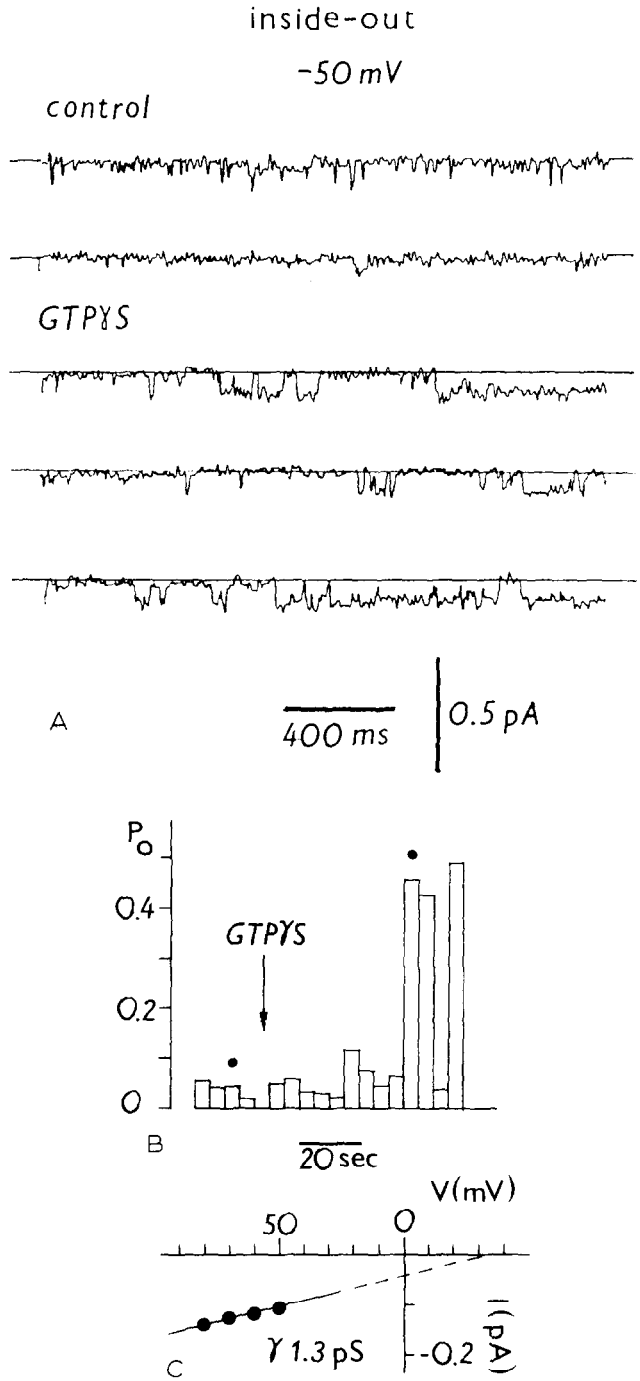

Fig. 2. Properties of SG-channels, inside-out experiment. (A) Current records at −50 mV, filter 0.1 kHz. (B) Time course of the channel activity before (shown only last 20 s stretch) and after the GTPγS (100 μM) application. (C) Unitary current/voltage relation.

significantly higher than in experiments without EGF (see below). After the patch had been excised the channel activity subsided and resumed again on the application of non-hydrolyzable analogue of GTP, GTPγS. Similar Ca-permeable channels responsive to analogues of GTP were oberved in 24 inside-out experiments. Their conductances were from 1.9 to 2.6 (mean 2.27 pS) at 20–23°C and from 2.0 to 2.9 (mean 2.39 pS) at 30–33°C. The channels with conductances within these limits were observed in 73 cell-attached and inside-out patches; we assume that all of them belong to the same G-type.

To quantify the effect of channel activators we calculated mean P_o values over 20 s interval ($P_{o(20)}$) when channel activity was maximum for each condition (absence or presence of an activator). GTP analogues increased $P_{o(20)}$ from 0.019 ± 0.004 to 0.19 ± 0.03 (mean ± SE, $n = 24$). In cell-attached patches mean $P_{o(20)}$ values were 0.018 ± 0.005 (17) and 0.17 ± 0.05 (16) without and with EGF in the pipette, respectively.

Fig. 2 shows the experiment on a cell-free patch containing a single channel of another type. As with G-channels, the currents through this channel are carried by Ca^{2+} rather than anions since their amplitudes were indifferent to whether glutamate$^-$ or Cl^- was a major anion in the intracellular solution. The current/voltage relation shows slope conductance of 1.3 pS and extrapolated reversal potential +30 mV. Channels with slope conductances between 1.2 and 1.4 pS, indifferent to anion species in the intracellular solution were observed in 8 experiments. We assume that all of them belong to the same type referred to as SG-type.

It can be seen from Fig. 2 that the addition of GTPγS causes a large increase in the channel openings. The channel activity measured in $P_{o(20)}$ values increased 10 times in this experiment. In other inside-out experiments GTPγS or GppNHp (100 M) increased $P_{o(20)}$ of SG-channels from 0.02 ± 0.01 to 0.38 ± 0.07 ($n = 6$).

The effect of GTP analogues on the activity of G- and SG-channels suggests that G proteins [11] mediate the activation of these channels by EGF and other extracellular agonists.

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