Possible involvement of GTP-binding proteins in the deactivation of an inwardly rectifying K⁺ current in enterocytes isolated from guinea-pig small intestine

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Abstract. The possible regulation of guinea pig enterocyte ion channels by GTP-binding proteins has been investigated by using the whole-cell recording mode of the patch-clamp technique and non-hydrolysable analogues of GTP. The main K⁺ currents in these cells are mediated by inwardly-rectifying K⁺ channels. Intracellular dialysis with hydrolysis-resistant GTP analogues leads to the deactivation of the inward K⁺ currents. Non-hydrolysable analogues of ATP or GDP were without effect. The effect occurs after a lag phase of 2 to 7 min, suggesting a multistep mechanism. Cl⁻ currents were not affected by any of the nucleotide analogues. It is suggested that inwardly-rectifying K⁺ currents are deactivated by a G-protein-dependent process.

Keywords: K⁺ channels, inward rectification, G-protein, guinea pig enterocyte

Introduction

Enterocytes from the small intestine possess K⁺ channels that are thought to be regulated in order to provide an exit pathway for extra K⁺ influx occurring during increased absorption or secretion. In Necturus enterocytes this pathway may be provided by Ca²⁺-activated K⁺ channels (Sheppard et al., 1988). The K⁺ conductance of guinea-pig villus enterocytes, however, has recently been shown to be dominated by inwardly rectifying K⁺ channels with little contribution from Ca²⁺-activated K⁺ channels (Fargon et al., 1990a). This type of ionic channel might provide the means of coupling the uptake of K⁺ through the Na⁺-K⁺-pump to its recycling through the basolateral membrane. A variety of bacterial toxins and neurohumoral agents produce Cl⁻ secretion. There is also evidence that Na⁺ absorption is inhibited during the action of these secretagogues in the small intestine (Donowitz & Welsh, 1987); such an action might be exerted through an inhibition of K⁺ channels and, hence, of basolateral recycling of K⁺ taken up through the Na⁺ pump. We report here that the inward K⁺ rectifier of guinea-pig villus enterocytes can be efficiently deactivated by intracellular, non-hydrolysable analogues of GTP. In the same experiments Cl⁻ currents are not affected. It is suggested that this type of modulation might have consequences for transepithelial ion transport and might be involved in the action of secretagogues.

Materials and methods

The cell isolation method developed by Del Castillo (1987) was followed with only minor modifications. The villus origin of these cells was checked by measuring their sucrase activity.

Whole-cell currents were recorded by the patch-clamp methodology according to Hamill et al. (1981). The composition of bath and pipette solutions is given in the legends to figures. Cells were clamped at a holding potential (typically -40 mV) and membrane currents in response to depolarising and hyperpolarising voltage steps measured. Voltage pulse protocols and data acquisition were carried out simultaneously using an IBM AT microcomputer with a LabMaster laboratory interface and software developed by one of us (F.F.). Digitalisation rate was usually 5 kHz. To avoid the capacitance surge, 1 ms at the beginning of the voltage-clamp pulses transitions is omitted from the current traces.

ATP-γ-S, adenosine 5′-O-(3-thiotriphosphate); EGTA, ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid; GDP-β-S, guanosine 5′-O-(3-diphosphate); GMPPNP, 5′-guanylyl imidodiphosphate; ATP, guanosine 5′-triphosphate; GTP-γ-S, guanosine 5′-O-(3-thiotriphosphate); TPA, 12-O-tetradecanoylphorbol 13-acetate were all purchased from Sigma Chemical Company, Poole, Dorset.

Results

Initial experiments were conducted with pipette and bath solutions containing low Cl⁻, in order to eliminate Cl⁻ currents, and with 16 mM K⁺ in the extracellular solution. Under these conditions the dominant current is a K⁺ inwardly rectifying current (Fargon et al., 1990a). The experiment shown in Fig. 1 was carried out with a pipette solution containing 100 μM GTP-γ-S. The currents elicited by the voltage protocol given in A soon after breaking into
the cell are shown in B. Large inward currents were observed which activated within 5-10 ms and with strong hyperpolarisations showed partial inactivation. Very little outward current was elicited by depolarising voltages, owing to the absence of Cl', which is the only other significant charge carrier in these cells (Fargon et al., 1990b). In Fig. 1E the current-voltage relationship for the currents at the peak of activation is shown. The inward current, which has been shown to be a K+ current (Fargon et al., 1990a), has an extrapolated reversal potential of -47 mV, which is close to EK under the conditions used (-54 mV). The slope conductance was 18 nS. Fig. 1C and D show voltage clamp sequences taken at 4 and 6 min after the establishment of the whole-cell recording configuration. A progressive reduction of the inward currents was seen. The corresponding current-voltage relationships show that while the reversal potential remained fairly constant, the conductance decreased to 17, 12 and 1 nS at 2, 4 and 6 min respectively.

Figure 2 illustrates the time course and cell to cell variability of the effect. Here cells were subjected to pulse trains consisting of a 100 ms hyperpolarisation to -140 mV followed by a return to the holding potential for 200 ms and then a 100 ms depolarising pulse to 100 mV. These pulse trains were separated by 20 s intervals during which the cell was held at -40 mV. The pipette contained a K gluconate-rich solution while the bath solution was Cl-rich and contained 16 mM K+. Under these conditions inward current measured at -140 mV is carried by K+ while the outward current measured at 100 mV is carried by Cl'. Fig 2 shows experiments performed with GMPPNP (A) or GTP-γ-S (B) in the pipette. Cl' currents were very little affected by dialysis with the GTP analogues while K+ currents were practically abolished in times ranging from 2-7 min after the establishment of the whole-cell configuration. The

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Fig. 1. Effect of intracellular GTP-γ-S on inward K+ currents. Currents elicited by the voltage protocol shown in A were recorded immediately (B), 4 min (C) and 6 min (D) after the establishment of the whole-cell configuration. In E the current-voltage relationships are shown; measurements were taken at the steady-state or at the peak for the most hyperpolarised pulses. The pipette solution was (mM): 146 K gluconate, 5 EGTA, 5 MgCl2, 1 Na2ATP, 0.1 GTP-γ-S, 10 HEPES, pH 7.2. Bath solution was: 5 KCl, 0.36 K2HPO4, 1.3 CaCl2, 0.44 KH2PO4, 0.5 MgCl2, 4.2 NaHCO3, 130 Na gluconate, 10 K gluconate, 10 HEPES, pH 7.2. The holding potential (Vh) is given.

Fig. 2. Time course of deactivation of K+ currents by 100 μM GTP-γ-S or GMPPNP. The solutions used were those given in the legend to Fig. 1, except that all gluconate in the bath solution was replaced by Cl'. The cell was held at -40 mV except for 100 ms square pulses to -140 or 100 mV given at 20 s intervals. A: results with GMPPNP, B: results with GTP-γ-S. Different symbols refer to different cells. Results are normalised to the absolute value of the currents observed with the first voltage train.