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Hormonal regulation of pituitary GH_3 cell K^+ channels by G_k is mediated by its α-subunit

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The resolved α-GTPγS (α*) and $\beta\gamma$-subunits of human erythrocyte G_k, the stimulatory regulatory component of hormone-responsive K^+ channels, were tested for their potential stimulatory activities on the K^+ channel of the endocrine GH_3 cell. Concentrations as low as 0.5 pM α_k* consistently activated K^+ channels in isolated membrane patches, and saturating effects were obtained with 50 pM α_k*. In contrast 2000–4000 pM $\beta\gamma$ was without effect. We conclude that G_k acts on K^+ channels through its α-subunit in a manner akin to that of G_s acting on adenylyl cyclase and transducin acting on cGMP-specific phosphodiesterase of photoreceptor cells.

G-protein subunit; Ligand-gated K^+ channel; Somatostatin receptor; Muscarinic receptor; Transmembrane signaling; Coupling protein; (Endocrine cell)

1. INTRODUCTION

G-proteins are heterotrimers of composition $\alpha\beta\gamma$. They bind and hydrolyze GTP, and mediate effects of hormone and neurotransmitter on effector systems such as adenylyl cyclase, phospholipase C, cGMP-specific phosphodiesterase (cG-PDE) and, a so-called receptor-gated K^+ channel [1,2] present in many cells including heart atrial and pituitary GH_3 cells. On activation with non-hydrolyzable GTP analogs purified G-proteins dissociate into an α-G nucleotide complex plus a $\beta\gamma$ dimer. This reaction is thought to occur also in intact membranes under the control of GTP and hormonally occupied receptors. For stimulation of adenylyl cyclase and cG-PDE, the respective G-proteins involved, G_s and transducin (T), act through their respective α-subunits [3,4]. For inhibition of adenylyl cyclase, there are conflicting data as to whether G_i, the G-protein responsible for this, exerts its action through its α_i-subunit or its $\beta\gamma$-dimer, the latter acting either by preventing activation of G_s [5,6] or inhibiting directly the catalytic unit of the enzyme [7]. For G_k, responsible for activation of receptor-gated K^+ channels, the data are also conflicting as to which of its subunits mediates the regulatory event. We have shown that 2–20 pM GTPγS-activated G_k (G_k*), but not 2000 pM GTPγS-activated G_s (G_s*), is a potent activator of both heart [1] and GH_3 [2] cell K^+ channels in isolated membranes studied under patch-clamp conditions in the inside-out configuration as in [8]. Since G_s and G_k purified by us from human erythrocytes share the same $\beta\gamma$-subunits, we inferred that G_k acts through its α-subunit. On the other hand, performing similar experiments, Logothetis et al. [9] found no effect with two bovine brain α-subunits, but obtained activation of K^+ channels with a $\beta\gamma$ preparation of the same origin. Here we show, that for mammalian K^+ channels such as found in GH_3 cells, α-GTPγS, resolved from $\beta\gamma$, is a very potent K^+

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channel activator while $\beta\gamma$ resolved from α_k is inactive in doing so.

2. MATERIALS AND METHODS

G_k (80 μg), initially referred to as putative G_i (N_i), the inhibitory regulatory component of adenylyl cyclase [11] – later simplified to G_i (N_i) – purified as in [11,12] was treated with 100 μM GTPγS, 100 mM $MgCl_2$ and 150 mM NaCl in the presence of 5% Lubrol-PX and buffer A (1 mM EDTA, 20 mM β-mercaptoethanol, 10 mM Na-Hepes, pH 8.0, 30% (v/v) ethylene glycol) and applied after a 13-fold dilution with buffer A to 0.1 ml packed DEAE-Toyopearl (DEAE-Fractogel TSK 650, Pierce). The column was washed with buffer B (7 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10 mM Tris-HCl, pH 8.0), buffer B plus 60 mM NaCl and buffer B plus 200 mM NaCl. α_k-GTPγS, eluted in the last wash, was dialyzed extensively against buffer B plus 40 mM KCl and used without further treatments. Lubrol-PX present in purified human erythrocyte $\beta\gamma$ preparations [12] was reduced by applying it to a 0.1 ml DEAE-cellulose column and eluting the protein in a single step with 0.1% Lubrol-PX and 150 mM NaCl in buffer A. These preparations (200–400 μg/ml $\beta\gamma$) were used without further treatments. Other methods were as in [1,2], except that the solution in the patch pipettes was 130 mM KOH, 130 mM methanesulfonate, 5 mM EGTA, 1 mM $MgCl_2$ and 5 mM Hepes-Tris (pH adjusted to 7.2 with Tris base). The bath solution was the same plus 2 mM ATP and 100 μM cAMP. Other additives are indicated below.

3. RESULTS AND DISCUSSION

DEAE-Toyopearl chromatography of activated G_k yielded ~20% of the starting α_k-GTPγS complex (α_k*) and essentially none of the $\beta\gamma$-dimer. On analysis by SDS-polyacrylamide gel electrophoresis followed by Coomassie blue and silver staining, α_k* proved to be ~40 μg/ml (1 μM) and contaminated with a small amount (~1%) of $\beta\gamma$ (not shown). α_k* is very potent in stimulating opening of K^+ channels: as little as 0.5 pM led consistently (5 out of 5 trials) to K^+ channel opening, 5 pM was more effective. Almost continuously stimulated channels were obtained with 25–50 pM of the preparation (fig.1a). As with G_k* [2], channel openings occurred in bursts and clusters of bursts. The effect of increasing concentrations of α_k* was noted not only in the frequency of the openings within a burst, but also in an increase in the frequencies with which bursts occurred, leading to an increased frequency of simultaneous openings of more than one channel. Single-channel slope conductances of individual openings were 50–55 pS, and their mean open times were between 1.3 and 1.7 ms, which on the average are the same as obtained with G_k* [2].

In contrast to the effectiveness with which the preparation containing α_k* caused opening of K^+ channels in GH_3 cell membranes, we obtained no effect on addition of 3.6 nM $\beta\gamma$. The dimers were added following a dilution scheme in which the stock solution of $\beta\gamma$ is first diluted 80-fold in pipette buffer plus 0.1% bovine serum albumin and this solution is diluted 10-fold into the bath

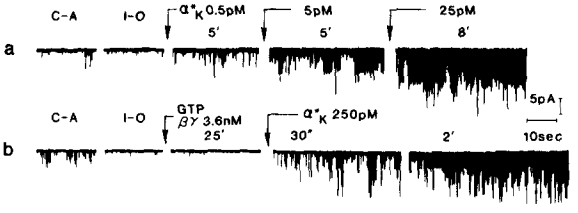

Fig.1. Effects of human erythrocyte α_k* and resolved human erythrocyte $\beta\gamma$-subunits on K^+ channel activity in excised inside-out membrane patches of adult guinea pig atrial cells. Clonal rat pituitary GH_3 cells were grown as in [2] on cover slips and K^+ currents were recorded by patch clamp techniques [8] in the cell-attached (C-A) and excised inside-out (I-O) modes using symmetrical isotonic K^+ (140 mM) solutions as described by Yatani et al. [1]. The holding potential was −80 mV, and additions were made to bathing solutions that did (panel b) or did not (panel a) contain 100 μM GTP throughout the whole course of the experiment. (a) Effect of increasing concentrations of α_k* added at 25 min intervals cumulatively in 10 μl aliquots to the 100 μl experimental chamber [1,2]. (b) Lack of effect of 3.6 nM $\beta\gamma$ on K^+ channels in an α_k*-responsive membrane patch. α_k* was added 30 min after $\beta\gamma$. Times at top of each trace are in minutes (') or seconds ('') elapsed between the preceding addition and the recording of the trace shown. The first additions were made between 7 and 10 min after excision of the patch. Calibration bars refer to both panels. Other conditions were as in [1,2].

solution in which the membrane patch is held. This insured reduction of the detergent to levels below its critical miscelle concentration while maintaining $\beta\gamma$ in solution. Fig.1b shows one of 3 experiments with a membrane patch held in a bath solution with 100 μM GTP. The same was obtained when GTP was omitted from the bath solution. Patches not responding to $\beta\gamma$ were all responsive to α_k* (fig.1b).

The report of Logothetis et al. [9] was unexpected in the light of data presented here and its results are puzzling. Our data indicate clearly that G_k acts on K^+ channels through its α-subunit, yet Logothetis et al. [9] saw no effects with bovine brain α-subunits. Explanations for this may be that the α-subunits purified from brain are not the correct ones, i.e. that neither brain α_{41} nor brain α_{39} is an α_k, or that as isolated the brain α-subunits had lost their ability to affect K^+ channels, or that their activation protocol was ineffective in promoting formation of α-GTPγS complexes. On the other hand, our experiments revealed no effect of $\beta\gamma$ dimers. Tests for contaminating holo-G protein in our $\beta\gamma$ preparation obtained from human erythrocytes, using [^{32}P]ADP-ribosylation with pertussis toxin, were negative at the 1 in 10000 level. Yet such a control was not carried out by Logothetis et al. who obtained channel opening on addition of 23 nM bovine brain $\beta\gamma$. Two explanations may apply: (i) $\beta\gamma$-dimers do indeed stimulate K^+ channels, but do so at concentrations 3–4 orders of magnitude higher than α_k; and (ii) the bovine brain $\beta\gamma$ preparations of [9] were contaminated by holo-G_k activated by the mixture of NaF-$AlCl_3$-$MgCl_2$ used to stabilize the proteins during their purification [9]. Special studies will be needed to settle the question as to whether $\beta\gamma$-dimers do indeed affect the receptor-gated K^+ channels. From the data presented here, it is clear, however, that the effects of receptors on these channels are mediated by the α-subunits of G_k.

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