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MINI-REVIEW

Control of K^+ Channels by G Proteins

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Abstract

Heterotrimeric G[3] proteins are thought to couple receptors to ionic channels via cytoplasmic mediators such as cGMP in the case of retinal rods, cAMP in the case of olfactory cells, and the cAMP cascade in the case of cardiac myocytes. G protein-mediated second messenger effects on K^+ channels are dealt with elsewhere in this series. Recently, membrane-delimited pathways have been uncovered and an hypothesis proposed in which the α subunits of G proteins directly couple receptors to ionic channels, particularly K^+ channels. While direct coupling has not been proven, the membrane-delimited nature has been established for specific G proteins and their specific K^+ channel effectors.

Key Words: G proteins; K^+ channels; pertussis toxin; membrane-delimited pathways; muscarinic receptor.

Introduction

Direct coupling between a G protein and an ionic channel seemed most likely for a muscarinic M2 receptor and a K^+[ACh][4] channel. The experimental results pointing to this possibility were: (1) the latency after ACh[5] application was about 100 msec (Purves, 1976; Glitsch and Pott, 1978; Hartzell, 1980; Osterrieder *et al.*, 1982), which was much longer than the latency at the

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[3] Guanine nucleotide binding, G.
[4] Muscarinic K^+, K^+[ACh].
[5] Acetylcholine, ACh.

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nicotinic acetylcholine receptor (1 msec) where the receptor and the ionic channel were the same protein; (2) the rate-limiting step was not agonist occupancy (Nargeot *et al.*, 1983); and (3) possible second messengers such as cGMP (Nawrath, 1977; Trautwein *et al.*, 1982; Nargeot *et al.*, 1983), cAMP (Trautwein *et al.*, 1982; Nargeot *et al.*, 1983), and Ca^{2+} (Trautwein *et al.*, 1982) were ruled out. Subsequently, it was shown that when ACh was applied outside a cell-attached patch of membrane it could not activate K^+ channels whereas ACh in the patch pipette could (Soejima and Noma, 1984). From binding studies, it was known that muscarinic M2 receptors were linked to G proteins in the heart and that PTX[6] substrates were present in atrial tissues (Rosenberger *et al.*, 1979; Hazeki and Ui, 1981; Kurose and Ui, 1983; Halvorsen and Nathanson, 1984). The link with electrophysiological was established when block of muscarinic effects on inwardly rectifying whole cell current and resting membrane potential by PTX was shown along with a requirement for intracellular GTP[7] (Pfaffinger *et al.*, 1985; Endoh *et al.*, 1985; Sirota *et al.*, 1985). In addition, the nonhydrolyzable GTP analog Gpp(NH)p disconnected the currents from ACh control (Breitwieser and Szabo, 1985). These experiments were complicated by the fact that a non-PTX sensitive phosphoinositide pathway (Brown and Brown, 1984) was also present in atrial cells and may have contributed inward current to the whole cell current or membrane potential measurements (Tajima *et al.*, 1987). A revealing set of experiments showed that GTPγS[8] applied to the cytoplasmic face of an inside-out membrane patch activated a specific set of inwardly rectifying, single K^+ channel currents that were the same as those activated by ACh and did so in an Mg^{2+}-dependent manner (Kurachi *et al.*, 1986a, b). The particular G proteins involved to this point had not been specified nor had a membrane-associated G protein effect such as protein kinase C activation been excluded. In our experiments, specific G proteins from natural membranes were applied to inside-out patches for observation of their effects. In addition, testing was done to determine whether protein kinase C activation could be involved (Yatani *et al.*, 1987a; Codina *et al.*, 1987b). These experiments established that a G protein, probably G_i-3 or its α subunit, when preactivated with GTPγS activated single channel K^+[ACh] currents in precisely the same manner as physiological activation. The effects occurred in the presence of AMP-PNP, and phorbol ester in the presence of ATP[9] had no effect. Phosphorylation by protein kinase C could, therefore, be excluded. Picomolar concentrations were effective whereas nanomolar concentrations of GTPγS were required to activate the responsible endogenous G protein.

[6] Pertussin toxin, PTX.
[7] Guanosine triphosphate, GTP.
[8] The nonhydrolyzable GTP analog guanosine 5′-O-(3-thiotriphosphate), GTPγS.
[9] Adenosine triphosphate, ATP.

Role of G Protein Subunits

Adenosine diphosphate-ribosylation with PTX blocked muscarinic activation and the response was reconstituted by unactivated G_i-3 in the presence of GTP. Thus, endogenous G_k cannot be tightly coupled to the muscarinic receptor since adenosine diphosphate-ribosylation with PTX would, in this case, have led to permanent loss of receptor-G protein-K^+ channel coupling; nor can the endogenous G_k be tightly coupled to the K^+[ACh] channel since exogenous G_i^*-3 or α_i^*-3 activated the channel. While GTP plus the unactivated trimeric G_i-3 stimulated K^+[ACh] channels in the presence of agonist and PTX, GTP plus $\beta\gamma$ could not reconstitute the response. This finding plus the fact that α_i^*-3 and holo G_i^*-3 were equipotent indicated that α_i^*-3 mediated the effect. While it was originally proposed that $\beta\gamma$ was responsible, it is now agreed that α subunits are responsible (Logothetis *et al.*, 1988; Kim *et al.*, 1989). $\beta\gamma$ subunits activated phospholipase A_2 (Kim *et al.*, 1989) and arachidonic acid and its 5′-lipoxygenase metabolites (Kurachi *et al.*, 1989) activated these same atrial K^+ channels. However, this mechanism was not operative in muscarinic or purinergic stimulation of K^+[ACh] channels since eicosanoid pathway blockers had no effect on the agonist-induced responses (Kim *et al.*, 1989; Kurachi *et al.*, 1989). Furthermore, there were inconsistencies between the observations of Kurachi *et al.* (1989) and Kim *et al.* (1989) regarding the postulated mediation of $\beta\gamma$ effects via arachidonic acid release. The most noteworthy was that the effects of arachidonic acid and its metabolites observed by Kurachi *et al.* (1989) required the presence of GTP whereas the $\beta\gamma$ effects of Kim *et al.* (1989) occurred in its absence (Logothetis *et al.*, 1987). Recently it has been shown that bromphenacyl bromide, which blocked entry of arachidonic acid to downstream metabolism, had effects on ionic channels (Yatani *et al.*, 1990). Nordihydroguiaretic acid, which blocks 5′-lipoxygenase pathways, also had nonspecific effects (Yatani *et al.*, 1990). Furthermore, biochemical evidence for a 5′-lipoxygenase pathway was lacking in heart muscle (Hohl and Rosen, 1987).

In most cases where it has been investigated, effects of $\beta\gamma$ have been shown to be inhibitory through a mass action effect on the α subunit (Katada *et al.*, 1984a, b), although in the case of retinal PLA_2 a direct stimulatory effect for $\beta\gamma$ subunits has been proposed (Jelsema and Axelrod, 1987). Evidence for $\beta\gamma$ mediation of the pheromone receptor response in *Saccharomyces cerevisiae* has been summarized by Bourne (1989). In the retina the concentrations of $\beta\gamma$ were in the micromolar range, i.e., ca. 10^6-fold higher than those needed to obtain an effect with α_i-3 on K^+ channels (Codina *et al.*, 1987a, b) or on adenylyl cyclase (Northup *et al.*, 1983). In the atrial K^+ channel experiments nanomolar $\beta\gamma$ was used. The $\beta\gamma$ activator results were complicated because the zwitterionic detergent CHAPS used in the

experiments of Clapham and collaborators (Logothetis *et al.*, 1987, 1988) could by itself activate atrial K^+ channels (Kirsch *et al.*, 1988; Cerbai *et al.*, 1988) as well as other ionic channels (Kirsch *et al.*, 1988). It is known that ionic detergents may act upon Na^+ channels and other membrane proteins including PLA_2 (Pind and Kuksis, 1988).

The idea that $\beta\gamma$ subunits may be stimulatory to atrial K^+ channels has also been challenged by experiments which show that $\beta\gamma$ subunits, including the same ones used by Logothetis *et al.* (1987, 1988), inhibited these channels. In some of these experiments (unpublished data) the detergent Lubrol was used to suspend the $\beta\gamma$ subunits. However, while care was taken to avoid any direct effects of Lubrol, these can never be ruled out completely. More convincing were the observations that the same results occurred, i.e., inhibition of muscarinic atrial K^+ channels, when hydrophilic $\beta\gamma$ of transducin was used, which did not require detergents. Moreover, $\beta\gamma$ was found to be more potent as an inhibitor of channels in the absence of agonist (EC_{50} ca. 10 Pm), suggesting that $\beta\gamma$ subunits have as one role the suppression of agonist-independent background noise, lending further support to the idea that the inhibitory effect was physiologically relevant.

The experiments to this point dealt mainly with reconstitution of the K^+[ACh] response. To probe further functional aspects, a monoclonal antibody was used that bound to α but not $\beta\gamma$ subunits. Monoclonal antibody 4A bound to α_T and α_i-3 and was shown to block the light-induced transition of rhodopsin to metarhodopsin II (Hamm *et al.*, 1988; Deretic and Hamm, 1987). The results showed that monoclonal antibody 4A blocked the muscarinic M2 receptor effect and, furthermore, the block was irreversible only if endogenous G protein was activated. Only two conclusions were possible: (a) that $\beta\gamma$ was liberated at a time when the mAChR effect was blocked; or (b) that αGTP$\beta\gamma$ and $\beta\gamma$ were equipotent, which was not the case. Neither result was consistent with a role for $\beta\gamma$ in muscarinic M2 receptor activation of K^+[ACh] (Yatani *et al.*, 1988a).

While recombinant α_i-3, activated either by GTPγS or AlF^{4-}, did stimulate the K^+ channel, thus removing any doubt as to structural identity of the α subunit of G_k (Mattera *et al.*, 1989; Yatani *et al.*, 1988b), the other recominant forms of α_i, when tested for effects on atrial K^+ channels, proved to be equipotent (Yatani *et al.*, 1988b). This led us to test the native versions which were contaminated by each other at less than 5%. Native GTPγS-activated α_i-1 (α_i^*-1) and α_i-2 (α_i^*-2) were prepared from purified bovine grain G_i-1 and human erythrocyte G_i-2 as described earlier for α_i^*-3 and were tested for effects on atrial K^+ channels. The native type 1 and type 2 molecules were active at the same low concentrations as α_i^*-3 and therefore the results from the recombinant forms of the α_i molecules were valid (Yatani

et al., 1988b). Thus, at least with respect to atrial K^+ channel regulation, types 1, 2, and 3 of the G_i's are G_k isoforms.

Interaction between the $G_i\alpha$ Subunit and K^+[ACh] Channel

Little is known about the site of protein–protein interaction at which the α subunit activates the K^+ channel. Based on the assumption that an inactivating particle kept the K^+[ACh] channel closed, several protein-modifying agents including trypsin, papain, glyoxal, and phenylglyoxal that remove Na^+-channel inactivation were tested. Of the agents tested, only trypsin activated muscarinic K^+ channels and it did so irreversibly. Trypsin activation produced single-channel currents in which inward rectification, single-channel conductance, mean open time, and burst duration were indistinguishable from muscarinic activation. Trypsin was effective in the absence of muscarinic agonist or intracellular Mg^{2+} and guanosine 5′-triphosphate. Heat-denatured trypsin was ineffective and trypsin inhibitor blocked the effect. Because trypsin was known to inactivate G proteins, the effect was probably on the K^+ channel or a structure closely associated with it. Trypsin cleaves proteins at lysine or arginine residues, and the arginine-specific reagents, glyoxal and phenylglyoxal, did not activate K^+ channels. Our hypothesis is that trypsin disrupted an inhibitory gating mechanism that normally held the channel closed in the absence of activated G_k. The inhibitory gate was physically distinct from the gate that mediated bursting and contained at least one trypsin cleavage point located at a lysine residue accessible from the cytoplasmic surface of the cell membrane. The inhibitory subunit is, therefore, analogous to the γ subunit of cGMP PDE (Kirsch and Brown, 1989).

Direct G Protein Pathway to ATP-Sensitive K^+ Channels in Heart

K^+_{ATP}[10] current is thought to be regulated by G proteins but the pathways which couple receptor, G protein, and channel have not been defined (Ribalet *et al.*, 1989; Parent and Coronado, 1989). Regulation of K^+_{ATP} current in neonatal rat ventricular myocytes was, therefore, examined. Application of 0.1 mM ATP to the intracellular side of membrane patches reduced K^+_{ATP} channel activity and addition of GTPγS at 0.1 mM restored activity. Application of 0.1 mM intracellular GTP plus 10 μM extracellular adenosine or

[10] ATP-sensitive K^+, K^+_{ATP}.

100 nM N^6-cyclohexyladenosine had the same effect as GTPγS; hence, K^+_{ATP} channels may be coupled to adenosine receptors via G proteins. Gα subunits preactivated with GTPγS were applied to the cytoplasmic side of membrane patches, and we found that α_{i1}, α_{i2}, and α_{i3} mimicked the effect of GTPγS but not α_0 or G_s, suggesting that $G_i\alpha$ acted via a membrane-delimited pathway. It was proposed that adenosine receptor coupling may be important for activating K^+_{ATP} channels in ischemic muscle.

Direct G Protein Coupling to K^+ Channels in Clonal Pituitary Cells

A situation similar to that of the muscarinic atrial K^+ channels is the situation which existed for K^+ channels in GH_3 clonal rat anterior pituitary cells (Yatani *et al.*, 1987b; Codina *et al.*, 1987a) in that somatostatin inhibited secretion, reduced intracellular Ca^{2+} levels (Schlegel *et al.*, 1985), and produced cAMP-independent membrane hyperpolarization (Yamashita *et al.*, 1986). In addition, the ACh effect was the same, which may be attributed to the same type of muscarinic receptors as are present in heart. These results led us to believe K^+ channels were involved and ACh and somatostatin were found to activate the same subset of GH_3 K^+ channels (Codina *et al.*, 1987a, b; Yatani *et al.*, 1987a, b). Human RBC G_i^*-3 directly activated single K^+ channel currents as it did in heart, but the conductance of these K^+ channels from this rat anterior pituitary cell line was 55 pS (Yatani *et al.*, 1987b), unlike the conductance of 40 pS for atrial $K^+[G_k]$ currents (Yatani *et al.*, 1987a; Codina *et al.*, 1987b; Kirsch *et al.*, 1988). The rectifying properties of $K^+[G_k]$ channel were not fully determined because the recordings were complicated by the fact that GH_3 cells, unlike atrial myocytes, have a variety of outward K^+ currents. Regardless, the data indicated that the atrial and clonal pituitary K^+ channels were different. G_s^* had no effect on this K^+ channel and G_0^* effects were weak, possibly due to the presence of a G_k, both of which were the same in heart. The GTP-binding α subunit of G_i-3, not the $\beta\gamma$ complex, stimulated GH_3K^+ channels as it did muscarinic atrial K^+ channels.

Direct G Protein Gating of Neuronal K^+ Channels

A similar line of experiments to those described for cardiac and pituitary cells led us to the suspicion that the G protein G_0, the most common G protein in brain for which no known function had been adduced, might activate neuronal K^+ channels directly. Thus acetylcholine M_2, adrenergic α_2, serotonin, dopamine, adenosine, γ-aminobutyric acid, and opioid δ and

μ somatostatin receptors were coupled to whole cell neuronal K^+ currents by GTP-dependent, PTX-inhibitable mechanisms (North *et al.*, 1987; Mihara *et al.*, 1987; Andrade *et al.*, 1986; Trussell and Jackson, 1987; Gähwiler and Brown, 1985; Sasaki and Sato, 1987; Aghajanian and Wang, 1986; North and Williams, 1985; Yakel *et al.*, 1988; Moore *et al.*, 1988). The existence of several novel G_0-gated K^+ channels were established in cultural hippocampal neurons (VanDongen *et al.*, 1988). Application of purified bovine brain G_0^* to the cytoplasmic aspect of inside-out membrane patches activated four types of single-channel K^+ currents having slope conductances of 13, 38, 38, 55 pS. One set of 38-pS channels was inwardly rectifying, differed from the other in its burst behavior, and was a nonrectifying channel. The 38-pS inwardly rectifying channel was coupled to serotonin receptors, but the receptors to which the other G_0-activated K^+ channels are coupled, if any, are unknown. No such stimulatory effects were observed on these channels with human erythrocyte G_i^*-3 or α_i^*-3. The neuronal results contrasted with earlier observations using the same preparation of G_0^* added to guinea pig atrial membrane patches which showed only marginal effects. The hippocampal K^+ channels were highly sensitive to G_0^*, significant activation was obtained at 1 pM, and half maximal effects were obtained at about 10 pM. In a few cases involving as yet uncharacterized channels, it was observed that α_i^*-3 but not α_0^* was an activator.

To confirm that the channels to which G_0^* was added were indeed gated by G_0 and not by a contaminant, the G protein specificity of the channel was studied by addition of partially purified recombinant α_0^* molecules obtained by the pT7 expression method discussed below including not only those of α_0^* but also α_i-1, α_i-2, and α_i-3. All of the above-mentioned types of K^+ channels were stimulated by recombinant α_0^* under conditions where prior addition of one of the recombinant α_i^* preparations, active on guinea pig atrial muscarinic K^+ channels, had no effect. The G_0-gated channels were stimulated in the absence of Ca^{2+} or ATP, and the presence of AMP-P(NH)P, added routinely to inhibit K_{ATP}^+ pS K^+ channels, and EGTA did not interfere with the actions of G_0^* or recombinant α_0^*. Thus, in hippocampal pyramidal cells of the rat, G_0 is a G_k and the K^+ channels gated by it are several and differ from those present in atrial cells in various aspects including G protein specificity.

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