Participation of RGS8 in the ternary complex of agonist, receptor and G-protein

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Abstract

The RGS (regulators of G-protein signalling) protein family sharpen signalling kinetics through heterotrimeric G-proteins by enhancing the GTPase activity of the G-protein α subunit. Paradoxically, they also accelerate receptor-stimulated activation. We investigated this paradox using the cloned G-protein gated K⁺ channel as a reporter of the G-protein cycle, and FRET (fluorescence resonance energy transfer) between cyan and yellow fluorescent protein tagged proteins to detect physical interactions. Our results with the neuronal protein, RGS8, show that the enhancement of activation kinetics is a variable phenomenon determined by receptor type, G-protein isoform and RGS8 expression levels. In contrast, deactivation was consistently accelerated after removal of agonist. FRET microscopy revealed a stable physical interaction between RGS8-yellow fluorescent protein and Gα, cyan fluorescent protein that occurred in the presence and absence of receptor activation and was not competed away by Gβy overexpression. FRET was also seen between RGS8 and Gγ, demonstrating that RGS8 binds to the heterotrimeric G-protein as well as G-protein α subunit-GTP and the transition complex. We propose a novel model for the action of RGS proteins on the G-protein cycle involving participation of the RGS in the ternary complex: for certain combinations of agonist, receptor and G-protein, RGS8 expression improves upon the ‘kinetic efficacy’ of G-protein activation.

Introduction

Numerous extracellular signals (hormones, neurotransmitters, odorants, photons) act at seven transmembrane receptors that couple with heterotrimeric G-proteins composed of α and a complex of β and γ subunits. The duration of the intracellular signal is dictated by the lifetime of the activated G-protein. Activation occurs in response to binding of an agonist to its target receptor, which stimulates the exchange of GDP for GTP on the Gα (G-protein α subunit), followed by dissociation of the Gα and Gβγ subunits and subsequent activation of their downstream effectors, which include enzymes and ion channels [1]. Signal termination occurs due to the intrinsic GTPase activity of the active Gα-GTP subunit that reverts to inactive Gα-GDP, which then reassembles with the Gβγ subunit, preventing interaction with their respective effectors.

The RGS (regulators of G-protein signalling) protein family, first identified in the 1980s as negative RGS in yeast [2], now contains at least 30 mammalian members. Each has a conserved RGS domain of 120–130 amino acids that is capable of binding to active Gα-GTP and accelerating the GTP hydrolysis rate 100–1000-fold [3]. The conserved RGS domain is flanked by N- and C-termini of varying lengths.

Some RGS proteins contain a myriad of other protein domains that have been implicated in mediating RGS target specificity [4], scaffolding functions [5] and cross-talk with other signalling pathways [6]. In contrast, the R4 subfamily (RGS1–RGS5, RGS8, RGS13 and RGS16) have short, amphipathic helical N-termini and featureless C-termini. These RGS proteins are generally considered prototypical as they appear to have little function other than to act as specific GAPs (GTPase-activating proteins) on either Gi/0 and/or Gq/0.

RGS effects on the activation of the G-protein gated K⁺ channel

Typically, RGS action exerts an inhibitory effect on signalling pathways by expediting Gα inactivation, thereby facilitating reassembly of Gα and Gβγ [3,4]. In bizarre contrast, expression of the R4 subfamily of RGS proteins has been found to accelerate receptor activation of the GIRK (G-protein-gated inwardly rectifying K⁺) channel [7–9]. The GIRK channel is directly gated by Gβγ subunits that are released from pertussis toxin (PTx)-sensitive Gi/0 G-proteins [1,10]. As predicted, the deactivation of the channel was significantly accelerated by RGS proteins on removal of agonist, but the signal amplitude during an agonist application was not considerably decreased and activation kinetics were enhanced [7–9]. This presented a conundrum for which the most obvious interpretation is that the RGS protein aids in the activation of the G-protein, in addition to catalysing the ‘turn-off’ reaction, possibly by scaffolding the G-protein at the GPCR (G-protein-coupled receptor) in a pre-formed complex.
complex [7]. Alternatively, by virtue of its GAP activity, the RGS protein may prevent GPCR/G-protein dissociation during agonist stimulation, allowing rapid G-protein re-activation and maintaining the signal amplitude by a ‘kinetic scaffolding’ mechanism [3,11].

Results

To explore these and other hypotheses, we focused on a member of the R4 subfamily, neuronal RGS8, which was tagged with YFP (yellow fluorescent protein). The effects of RGS8-YFP were assayed in pre-established HEK-293 (human embryonic kidney 293) cell lines stably expressing the GIRK channel subunits (Kir3.1/3.2A) plus one of a number of G_{i/o}-coupled GPCRs. The main finding was that activation of GIRK channel through two of the GPCRs, the adenosine A_{1} and adrenergic α_{2A}, was intrinsically fast and not enhanced by high-level expression of RGS8-YFP. Conversely, through the muscarinic M_{4}, dopamine D_{3} and heterodimeric GABA_{B} (γ-aminobutyric acid) receptors, G-protein activation was much slower and was significantly accelerated by RGS8-YFP expression. The A_{1}, α_{2A} and D_{2} receptors were expressed at very similar levels in their respective HEK-293 cell lines [12]. These effects were independent of the N-terminus of RGS8 because expression of a ΔN-RGS8-YFP construct yielded identical kinetic effects with those of RGS8-YFP [13]. Therefore the N-terminus of RGS8 did not prevent the assembly of RGS8 with certain GPCRs (e.g. A_{1} and α_{2A}) and was not necessary for an association with the other group of GPCRs (GABA_{B}, D_{2} or M_{4}). Furthermore, the preferential action of RGS8 was not found to lie at the level of the G-protein [13]. When signalling was constrained to transfected PTx-insensitive G_{o/α} (C-G) subunits in the A_{1} and GABA_{B} cell lines (in PTx-treated cells), RGS8-YFP accelerated GABA_{B}-mediated current activation but not A_{1}-mediated activation of currents [13]. Clearly, it is not a preferential action on certain Gα isoforms that dictates the behaviour of RGS8, but the combination of receptor with G-protein that is important. A clue to the target of RGS8 selectivity came from experiments to ensure that RGS8-YFP could interact functionally with CFP (cyan fluorescent protein)-tagged G_{o/α} subunits. G_{o/α}-CFP was rendered PTx-resistant by a single amino acid mutation at the C-terminus, which diminishes the affinity of the GPCR for the G-protein [12]. Recall that the α_{2A} receptor was intrinsically fast to activate current through the endogenous G-proteins of the cells. When constrained to signal through G_{o/α}-CFP in PTx-treated cells, currents initiated at the α_{2A} receptor showed retarded activation kinetics for which activation and deactivation were significantly accelerated by RGS8-YFP. This observation argues for a role of RGS8 in the ternary complex of agonist, GPCR and G-protein. Where the ternary complex is strong, it exhibits high ‘kinetic efficacy’ in G-protein activation with no acceleration by an RGS, but where the ternary complex is weak, or artificially weakened by constraining signalling through a mutant Gα construct, RGS8 appears to improve its kinetic fidelity.

The importance of RGS8 concentration in GIRK-activation kinetics was then explored. At low levels RGS8 merely acted catalytically, enhancing the ‘turn-off’ reaction. The acceleration of G-protein activation was achieved only at very high-level RGS8 expression [13]. These results are consistent with the concept that at high concentrations of RGS8, stable complexes are precipitated, possibly in a 1:1 stoichiometry, with the Gα. To address the association of RGS8 with the ternary complex more directly, FRET (fluorescence resonance energy transfer) microscopy was used to monitor the proximity of RGS8-YFP and G_{o/α}-CFP. We found that stable complexes formed between these two species at the plasma membrane in living cells [14]. Furthermore, the FRET signal did not change in response to receptor activation of the G-protein. Indeed, a significant (albeit weaker) FRET signal was detected between RGS8-YFP and G_{γ}-CFP, demonstrating that RGS8-YFP associates with the G-protein heterotrimer, as well as with Gα-GTP and the transitional complex [14].

In such a complex, it is credible that the RGS protein may have a direct effect on G-protein activation, acting in concert with an activated receptor. In the crystal structure of GS4 bound to G_{α1}, the membrane-attached N-terminus of G_{α1} is rotated slightly with respect to the main body of G_{α1}, although the authors comment that this may be an artifact of crystal packing [15]. This rotation presents a broader G-protein membrane-facing expanse for interaction with the relatively narrow cytoplasmic face of a GPCR. Speculatively, high-level RGS expression may precipitate complexes with heterotrimeric G-proteins in the cell that biases them towards activation by dimeric receptors [16].

Our suggestion that such complexes may only form at higher concentrations may confine the attendant acceleration of G-protein activation kinetics to specialized microdomains where these components may be concentrated. As yet, this point has not been addressed directly by any experimental work. Indeed, it seems that RGS proteins are present at lower concentrations than G-proteins: RGS4 and RGS7 constituted only 0.001% and 0.003%, respectively, of rat brain protein [5], whereas a separate study found that G_{i/o} G-proteins made up 1.5% of the membrane protein [1]. However, several RGS mRNA and/or protein levels have been shown to be up-regulated by persistent activation of a GPCR by agonist and in disease states [4]. Potentially, therefore, RGS-mediated acceleration of activation kinetics may be a feature of the GIRK-signalling pathway under physiological conditions, such as at the synapse or in pathological states.

References


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