Activator of G-protein Signaling 1 Blocks GIRK Channel Activation by a G-protein-coupled Receptor

APPARENT DISRUPTION OF RECEPTOR SIGNALING COMPLEXES*

Received for publication, January 31, 2002
Published, JBC Papers in Press, February 12, 2002, DOI 10.1074/jbc.M201064200

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The Ras-related protein, activator of G-protein signaling 1 (AGS1) or Dextras1, interacts with G\(_{\alpha}\) and activates heterotrimeric G-protein signaling systems independent of a G-protein-coupled receptor (GPCR). As an initial approach to further define the cellular role of AGS1 in GPCR signaling, we determined the influence of AGS1 on the regulation of G\(_p\)-regulated inwardly rectifying K\(^+\) channel (GIRK) current (I\(_{GIRK}\)) by M\(_2\)-muscarinic receptor (M\(_2\)-MR) in Xenopus oocytes. AGS1 expression inhibited receptor-mediated current activation by >80%. Mutation of a key residue (G31V) within the G\(_1\) domain involved in nucleotide binding for Ras-related proteins eliminated the action of AGS1. The inhibition of I\(_{GIRK}\) was not overcome by increasing concentrations of the muscarinic agonist acetylcholine but was progressively lost upon injection of increasing amounts of M\(_2\)-MR cRNA. These data suggest that AGS1 may antagonize GPCR signaling by altering the pool of heterotrimeric G-proteins available for receptor coupling and/or disruption of a preformed signaling complex. Such regulation would be of particular importance for those receptors that exist precoupled to heterotrimeric G-protein and for receptors operating within signaling complexes.

Heterotrimeric G-proteins transduce a variety of extracellular stimuli into intracellular responses. Such stimuli are primarily sensed by the superfamily of G-protein-coupled receptors. In general, the specific intracellular response is determined by the external stimuli itself and cell type-specific expression of receptors, G-proteins, and effectors. However, additional proteins may also operate to influence signaling specificity as well as signal magnitude and duration. Such accessory proteins may act as scaffolding proteins within a signal transduction complex and/or directly influence the basal activation state of G-proteins and effectors independent of an activated GPCR\(^1\) (1–8). The latter proteins include both the family of regulators of G-protein signaling (RGS), initially defined based upon their ability to accelerate the GTPase activity of selected G\(_{\alpha}\) proteins, and the recently identified activators of G-protein signaling (AGS) 1–3.

AGS proteins (AGS1–3) were identified in a yeast-based functional screen as receptor-independent activators of G-protein signaling (1, 2). These proteins do not share any sequence homology, and each entity exerts different effects within the context of the G-protein activation/deactivation cycle (1–4). AGS1 (AF069506) selectively activated the G\(_{\alpha}\)-protein-signaling pathway, and it appears to act as a guanine nucleotide exchange factor for G\(_i\), somewhat mimicking a GPCR (1, 4). AGS1 is a member of the Ras superfamily of small G-proteins providing a potential interface between signaling pathways regulated by the two broad classes of G-proteins. AGS1 is the human counterpart of the Ras-related protein Dextras1 (NP_033052), which was identified as a dexamethasone-inducible cDNA in AIT-20 mouse corticortroph cells (9), where it may influence cAMP regulation of hormone secretion (10). AGS1 was also implicated in N-methyl-D-aspartate receptor signaling in neuronal cells, where it is an apparent target of neuronal nitric-oxide synthase (11). Thus, AGS1 is clearly involved in cellular signaling events and binds to as well as activates G\(_{\alpha}\), but it is not known how this apparent G-protein regulator influences the activation of signaling systems by a GPCR. AGS1 may influence the specificity, magnitude, or duration of GPCR signaling events as it may actually interfere with receptor-effector coupling (12) by altering the pool of G-protein available for interacting with receptor.

As an initial approach to define the role of AGS1 in GPCR signaling, we determined the influence of AGS1 on the regulation of G\(_{\beta\gamma}\)-regulated inwardly rectifying K\(^+\) channel (GIRK) current (I\(_{GIRK}\)) by M\(_2\)-muscarinic receptors (M\(_2\)-MR) in Xenopus oocytes (13, 14). AGS1 expression had little effect on basal levels of current (I\(_{GIRK}\)) but inhibited the increase in GIRK channel activity elicited by activation of the M\(_2\)-MR. The inhibition of I\(_{GIRK}\) by AGS1 was progressively lost upon injection of increasing amounts of M\(_2\)-MR cRNA. These data suggest that AGS1 antagonized GPCR signaling by altering the pool of heterotrimeric G-proteins available for receptor coupling and/or by disrupting a preformed signal transduction complex.

EXPERIMENTAL PROCEDURES

cRNA Synthesis—GIRK1, GIRK4, and M\(_2\)-MR in pcDNA3.1 vector (Invitrogen) were kindly provided by Drs. Paolo Kofuji and H. A. Lester (California Institute of Technology). AGS1 and AGS1G31V were propagated by guest on July 23, 2018http://www.jbc.org/Downloaded from

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1 The abbreviations used are: GPCR, G-protein-coupled receptor; AGS, activator of G-protein signaling; GIRK, G\(_{\alpha}\)-regulated inwardly rectifying K\(^+\) channel; M\(_2\)-MR, M\(_2\)-muscarinic receptors.
2 M. Cismowski and E. Duzic, unpublished observations.
AGS1 Blocks GIRK Channel Activation by Receptor

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**RESULTS AND DISCUSSION**

The ability of AGS1 to interact with heterotrimeric G-proteins raises immediate questions as to its role in signal processing following activation of a GPCR at the cell surface. To address this question, we investigated the role of AGS1 on M2-MR coupling to GIRK channels using a *Xenopus* oocyte expression system. The *Xenopus* oocyte expression system has been widely utilized to analyze the function and regulation of GIRK channel activities because of the ease of gene expression and functional readouts. In oocytes injected with GIRK1/4 cRNAs and M2-MR cRNA, an inward K⁺ current (I_hK) was elicited by eluting ND96 for a high potassium solution. Acetylcholine elicited an additional inward K⁺ current (I_ACh), and this receptor-mediated event was completely blocked by injection of pertussis toxin.

Co-injection of AGS1 with GIRK1/4 and M2-MR cRNAs elicited little change in I_hK but markedly diminished I_ACh (Fig. 1). Mutation of residues in the G1 domain of AGS1 (G31V) rendered AGS1 inactive (Fig. 1, A and B), as was the case for AGS1 in the yeast assay system and for AGS1 regulation of ERK activity in COS-7 cells (1, 4). The inhibition of I_ACh by expression of AGS1 was progressively enhanced by injection of increasing amounts of AGS1 cRNAs (Fig. 2). At the lower expression levels of GIRK channels (0.01 ng/oocyte), AGS1 inhibited I_ACh by 76 ± 4.2% (Fig. 2A). In oocytes expressing higher levels of GIRK1/4 (2 ng), M2-MR (0.1 ng), and AGS1 or AGS1G31V (5 ng) and processed for channel recordings as described under “Experimental Procedures.”

![Diagram](http://www.jbc.org/)
of GIRK channels, AGS1 inhibited \( I_{ACH} \) by 47 ± 7.7% (Fig. 2B). Expression of AGS1G31V did not alter \( I_{ACH} \) at either expression level of GIRK channels (Fig. 2), indicating that the inhibition of \( I_{ACH} \) was not because of altered expression of receptor or channels, per se. This thought is further supported by the absence of a decrease in \( I_{ACH} \) when AGS1 was coexpressed with GIRK channels. Thus, AGS1 likely inhibits \( I_{ACH} \) by interfering with the transfer of signal from receptor to G-protein or perhaps from G-protein to the channels. Similar conclusions regarding the action of AGS1 on GPCR signaling were reported by Graham et al. (12) for formyl peptide receptor activation of ERK1/2 kinases in COS-7 cells.

As AGS1 clearly interacts with G\(_i\)/G\(_o\) α subunits (1, 4), AGS1 may inhibit \( I_{ACH} \) by competing with M\(_2\)-MR for the available pool of G-proteins. This possibility was addressed by examining the dose-response curve for acetylcholine in the presence and absence of AGS1 and by determining the ability of increasing amounts of M\(_2\)-MR to overcome the AGS1-mediated inhibition. Under standard experimental conditions (cRNA/oocyte: M\(_2\)-MR, 0.1 ng; GIRK1/4, 2 ng for each; AGS1, 5 ng) increasing concentrations of acetylcholine did not overcome the inhibitory effect of AGS1 on \( I_{ACH} \) (Fig. 3A). In contrast to the influence of increasing amounts of agonist, the inhibitory action of AGS1 on \( I_{ACH} \) was overcome by increasing the levels of expressed receptor. Increased levels of receptor increased the amount of \( I_{ACH} \), at fixed amounts of GIRK channels, suggesting that under these experimental conditions receptor was somewhat rate-limiting or that at least G-proteins and effectors were not saturated.
levels of M2-MR expression (Fig. 3). By complexing with a defined population of G-protein–signal transduction complex may consist of receptor and G-protein, the formation of this precoupled complex, and as such, even high concentrations of agonist would not be able to activate the formation exhibiting high affinity for agonist. AGS1 suggests that, indeed, AGS1 competes with the M2-MR for the pool of available heterotrimeric Gαi/Go to activate GIRK channel. This may reflect an action of AGS1 to disrupt a preconfigured signal transduction complex that is required for agonist activity. Thus increasing concentrations of agonist could not overcome the inhibitory affect of AGS1 action. Such a configurered signal transduction complex may consist of receptor and G-protein, G-protein and GIRK, or perhaps all three entities (17–19). By complexing with a defined population of G-proteins, AGS1 may limit the ability of a receptor to act catalytically as it cannot access multiple G-proteins.

These thoughts likely have important implications for Gαi/Gαo-coupled receptors that exist in a precoupled state where the receptor is complexed with G-protein and “stabilized” in a conformation exhibiting high affinity for agonist. AGS1 may block the formation of this precoupled complex, and as such, even high concentrations of agonist would not be able to activate downstream effectors. Increasing the amount of receptors in the presence of AGS1 as opposed to agonist itself would allow more of the receptor population to exist in a precoupled state and thus effectively override the inhibitory effect of AGS1 as was indeed the case in the present study.

A flurry of recent reports indicates that multiple proteins interact with and/or regulate the activation state of heterotrimeric G-proteins. Although the role of these proteins in GPCR processing can be varied, they certainly offer unexpected avenues for manipulating the signaling system. Controlling the population of receptors precoupled with G-protein or the generation of signaling complexes may be a key mechanism for regulating the action of hormones and as such provide new pathways for therapeutics that mimic or disrupt specific signaling systems.

Acknowledgments—We appreciate the suggestions and input of Drs. Motohiko Sato and Joe Blumer in the Lanier laboratory.

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J. Biol. Chem. 2002, 277:13827-13830.
doi: 10.1074/jbc.M201064200 originally published online February 12, 2002

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