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Biochemistry

RGS2/G0S8 is a selective inhibitor of Gqα function

(regulator of G protein signaling/phosphoinositide hydrolysis/phospholipase C-β)

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ABSTRACT **RGS (*r*egulators of *G* protein *s*ignaling) proteins are GTPase activating proteins that inhibit signaling by heterotrimeric G proteins. All RGS proteins studied to date act on members of the Giα family, but not Gsα or G12α. RGS4 regulates Giα family members and Gqα. RGS2 (G0S8) is exceptional because the G proteins it regulates have not been identified. We report that RGS2 is a selective and potent inhibitor of Gqα function. RGS2 selectively binds Gqα, but not other Gα proteins (Gi, Go, Gs, G12/13) in brain membranes; RGS4 binds Gqα and Giα family members. RGS2 binds purified recombinant Gqα, but not Goα, whereas RGS4 binds either. RGS2 does not stimulate the GTPase activities of Gsα or Giα family members, even at a protein concentration 3000-fold higher than is sufficient to observe effects of RGS4 on Giα family members. In contrast, RGS2 and RGS4 completely inhibit Gq-directed activation of phospholipase C in cell membranes. When reconstituted with phospholipid vesicles, RGS2 is 10-fold more potent than RGS4 in blocking Gqα-directed activation of phospholipase Cβ1. These results identify a clear physiological role for RGS2, and describe the first example of an RGS protein that is a selective inhibitor of Gqα function.**

Many hormones, neurotransmitters, and sensory stimuli rely on G proteins to exert their actions on target tissues (1, 2). In their resting state, G proteins exist as heterotrimers ($\alpha\beta\gamma$) with GDP bound to Gα subunits. Agonist activation of linked receptors stimulates GTP/GDP exchange on Gα, and the GTP-bound form of Gα dissociates from G$\beta\gamma$ to regulate the activity of target effectors; signaling is terminated upon Gα-catalyzed hydrolysis of GTP and heterotrimer reformation. Thus, G proteins are molecular switches, and the magnitude and duration of the signaling events they regulate are dictated by the lifetime of the active GTP-Gα complex.

Recent findings demonstrate that G proteins interact directly with a newly appreciated family of regulatory proteins termed RGS (*r*egulators of *G* protein *s*ignaling). RGS proteins were first identified genetically as negative regulators of G protein signaling in lower eukaryotic organisms including yeast, *Aspergillus* and *Caenorhabditis elegans* (3–5), and to date >20 unique mammalian isoforms have been identified by molecular cloning techniques (5). Biochemical evidence indicates that RGS proteins block G protein function by acting as GTPase activating proteins (GAPs) to limit the lifetime of the active GTP-Gα complex (6–8) and/or as effector antagonists (9). The few RGS proteins characterized to date negatively regulate signaling by members of Gi or Gq family of G proteins, but not Gs or G12. RGS1, RGS3, RGS4, RGS5, RGS10, RGS-r/RGS16, RET-RGS1, and GAIP have all been shown to interact with one or more Giα family members in assays designed to measure direct binding, GTPase activity, and/or Gi-mediated signaling events (5–12). The Gq class of G proteins link cell surface receptors to activation of the β isoforms of PLC and inositol lipid signaling (1, 2, 13). RGS4 and the related RGS protein GAIP have been shown to act as GAPs for Giα1 and Gqα in *in vitro* reconstitution systems (9). RGS4 and, to a lesser extent, GAIP also block GTPγS-Gqα-directed activation of PLCβ in cell membranes or when reconstituted as purified proteins into phospholipid vesicles. Of these two RGS proteins, only RGS4 blocks receptor and Gq signaling when exogenously expressed in intact cells (14, 15). RGS3 has also been reported to attenuate inositol phosphate production when transfected into cells (16, 17).

Among the well-studied RGS proteins, RGS2 is unique because the G proteins and signaling pathways it regulates are unknown. RGS2 is a 211 amino acid (24.4 kDa) protein first identified by screening cDNA libraries prepared from activated human monocytes (18) and, although initially named G0S8, it was renamed when it was later found by sequence comparisons to contain the highly conserved 120 amino acid core domain characteristic of RGS proteins (5, 12). In contrast to what is known about other RGS family members, previous biochemical studies suggest that RGS2 is the only RGS protein that does not interact with Gi family members (19, 20). In support of this idea is the observation that RGS2 is a weak inhibitor of G protein-mediated activation of mitogen-activated protein kinase pathways (12). Taken together, these findings predict that RGS2 selectively interacts with Gα subunits distinct from Gi or its family members. We report that RGS2 interacts specifically with Gqα, but not other Gα subunits, and that it is a potent inhibitor of Gqα-directed activation of PLCβ. These results identify for the first time a clear physiological role for RGS2, and provide the first example of an RGS protein that is a selective regulator of Gqα function.

EXPERIMENTAL PROCEDURES

Purified Proteins. Histidine-tagged RGS2 protein was expressed in *Escherichia coli* (BL21(DE3)) from the pET19b plasmid containing a full-length human RGS2 cDNA (kindly provided by D.R. Forsdyke, Queen's University, Kingston, ON, Canada). Wild-type histidine-tagged forms of RGS2 and RGS4, and inactive mutant forms of RGS4 (E87A, N88A double mutant; N128A single mutant; S. Srinivasa, N.W., and K.J.B., unpublished results) were purified using immobilized Ni^{2+}-NTA affinity chromatography, essentially as described

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Abbreviations: GAP, GTPase activating protein; GTPγS, guanosine 5′-(3-*O*-thio)triphosphate; $InsP_3$, inositol 1,4,5-trisphosphate; NTA, nitrilotriacetic acid; PLC, phopholipase C.

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(7). The sources of G proteins used for GAP assays have been described (7). Recombinant histidine-tagged Gqα was prepared and purified as described (21). Baculoviruses encoding untagged Gqα, Gβ, and histidine-tagged Gγ subunits, and methods for the expression and purification of untagged Gqα from Sf9 cells were as described (22). Purified PLCβ1 was a generous gift of R. Ball and P. Sternweis (University of Texas Southwestern Medical Center, Dallas, TX).

Assay of RGS Binding to G Protein α Subunits. Assays used to detect the binding of histidine-tagged wild-type or mutant RGS proteins to either Gα subunits present in bovine brain membrane fractions, or to purified Gα subunits were performed essentially as described (7). Bovine brain membranes (0.5 mg protein) in buffer A (20 mM Na-Hepes, pH 8.0/500 mM NaCl/3 mM DTT/6 mM $MgCl_2$) containing 100 μM GDP, or 100 μM GDP, 30 μM $AlCl_3$, and 10 mM NaF, were incubated 30 min at 5°C with histidine-tagged RGS proteins (10 μg). Membranes were solubilized with 1% cholate, and detergent-soluble extracts obtained after centrifugation at 100,000 $\times$ *g* were added to Ni^{2+}-nitrilotriacetic acid (NTA) agarose beads equilibrated with buffer A containing 20 mM imidazole, 0.1% $C_{12}E_{10}$ and 10 μM GDP or 10 μM GDP, 30 μM $AlCl_3$, and 10 mM NaF. Bound proteins were eluted with 500 mM imidazole, resolved by SDS/PAGE, transferred to nitrocellulose membranes and detected by Ponceau S staining and Western blot analysis with antisera 856 (detects Goα and Giα family members), WO82 (Gqα), 584 (Gsα) and B860 (G12/13; kindly provided by W. D. Singer, University of Texas Southwestern Medical Center, Dallas, TX). In assays using purified proteins, G protein α subunits (110 ng) were incubated [20 min at 22°C for Goα, and 30 min at 30°C for Gqα in HEDL buffer (50 mM Na-Hepes, pH 8.0/1 mM EDTA/1 mM DTT/10% glycerol/0.025% $C_{12}E_{10}$), ref. 7] with 1 mM GDP, GTPγS, or with 1 mM GDP, 30 μM $AlCl_3$ and 10 mM NaF. These Gα subunits (110 ng) then were incubated with histidine-tagged RGS4 or RGS2 (330 ng) in 40 μl buffer B (50 mM Na-Hepes, pH 8.0/1 mM $MgCl_2$/20 mM imidazole/0.025% $C_{12}E_{10}$/10 mM 2-mercaptoethanol/10% glycerol/500 mM NaCl) containing the appropriate guanine nucleotides (1 mM) with or without 30 μM $AlCl_3$ and 10 mM NaF. RGS-Gα complexes were isolated and detected as described above.

GTPase Assays. Assays to measure a single round of hydrolysis of GTP to GDP catalyzed by Gα subunits were performed as described previously (7). Briefly, various purified G protein α subunits (100 nM in 800 μl) were incubated with [γ-^{32}P]GTP (0.1 μM, 20–30,000 cpm/pmol) in the absence of Mg^{2+}. Aliquots (50 μl) were removed 30 s before and 10 s after hydrolysis of GTP was initiated at 5°C by adding $MgSO_4$ (10 mM final concentration), unlabeled GTP (100 μM final concentration), and either a buffer control or recombinant histidine-tagged RGS4 or RGS2 (100 nM final concentration unless otherwise specified). The amount of ^{32}Pi released was determined by liquid scintillation spectrometry.

Preparation of NG-108 Cell Membranes and Measurement of PLC Activity in the Presence of RGS Proteins. NG-108 membranes were prepared as described (9). Confluent NG-108 cells were grown at 37°C in DMEM supplemented with 10% fetal bovine serum, 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine and harvested in buffer containing 50 mM Na-Hepes (pH 8), 1 mM EDTA, 150 mM NaCl, and 0.1 mM phenylmethylsulfonyl fluoride. Homogenates were prepared by nitrogen cavitation and centrifugation at 500 $\times$ *g* to remove nuclei and unbroken cells. Supernatant fractions were centrifuged at 100,000 $\times$ *g*, and membranes were suspended in buffer C (50 mM Na-Hepes, pH 7.2/1 mM EDTA/3 mM EGTA/5 mM $MgCl_2$/150 mM NaCl/2 mM DTT/0.1 mM phenylmethylsulfonyl fluoride) prior to storage at −80°C. Reconstitution of RGS proteins with NG-108 membranes was carried out as described (9) with minor modifications. NG-108 membranes (6.5 μg per assay) in 10 μl of buffer C were mixed with an equal volume of RGS4 or RGS2 in buffer D (50 mM Na-Hepes, pH 7.2/3 mM EGTA/100 mM NaCl/2 mM DTT/80 mM KCl). Membrane/RGS samples were incubated at 4°C for 30 min and mixed with 30 μl of sonicated phospholipid vesicles containing [^{3}H]phosphatidyl inositol 4,5-bisphosphate and phosphatidlyethanolamine (13), and 100 μM GTPγS in buffer D. Reactions were initiated by the addition of 10 μl of 9 mM $CaCl_2$ in buffer D, and assays were carried out for 30 min at 30°C. Reactions were stopped and the samples processed as described (13).

Reconstitution of RGS Proteins with Gqα and PLCβ1. Reactions were performed essentially as described with minor modifications (9). Assays were carried out in a final volume of 60 μl. Purified recombinant Gqα was activated with 1 mM GTPγS for 1 hr at 30°C in buffer C. Activated Gqα (10 μl; 6 nM) was mixed with the indicated amounts of RGS2 or RGS4 in 10 μl of buffer D. The Gqα/RGS sample was incubated for 30 min at 4°C and mixed with 10 μl of 9 mM $CaCl_2$ in buffer D. The reactions were started by the addition of 30 μl of sonicated phospholipid vesicles containing [^{3}H]phosphatidyl inositol 4,5-bisphosphate and phosphatidlyethanolamine and purified recombinant PLCβ1 (1 ng) in buffer D. Assays were carried out for 20 min at 30°C. Reactions were stopped and the samples processed as described (21).

RESULTS

Comparison of the G Protein Binding Selectivities of RGS2 and RGS4. We and others have shown previously that recombinant RGS2 does not bind Giα family members under conditions that favor Gα binding by other RGS isoforms (19, 20). We therefore investigated whether RGS2 binds Gα subunits other than Giα family members. Initial experiments examined the capacity of purified histidine-tagged RGS2 to bind native Gα subunits present in bovine brain membranes. As positive and negative controls we used wild-type RGS4 and mutant forms of RGS4 (E87A, N88A double mutant; N128A single mutant) that are defective in GAP activity and Gα binding (S. Srinivasa, N.W., and K.J.B., unpublished results). Brain membranes were treated appropriately to place G proteins in one of two states: (*i*) inactive (GDP-bound); and (*ii*) transition state mimic (GDP + AlF_4^--bound). Treated membranes were incubated with wild-type and mutant RGS proteins and detergent extracts were prepared. RGS-Gα complexes were bound to Ni^{2+}-NTA resin, eluted and detected by Ponceau S staining of blots (Fig. 1*A*) and by immunoblotting using antisera specific for different classes of Gα subunits (Fig. 1*B*).

Under conditions that induce the transition state of Gα subunits, wild-type but not mutant forms of RGS4 bound nearly stoichiometrically to a polypeptide of apparent molecular mass of 40 kDa (Fig. 1*A*). Western blot analysis experiments indicated that this 40-kDa polypeptide was predominantly a mixture of Goα and Giα. Similarly, wild-type but not mutant forms of RGS4 bound Gqα as detected by immunostaining with specific anti-Gq sera; Gqα was present at levels too low to be detected by Ponceau S staining (Fig. 1*B*). By comparison, RGS2 failed to bind either Goα or Giα. However, RGS2 did bind Gqα with an apparent efficiency similar to that of wild-type RGS4 (Fig. 1*B*). In separate experiments carried out under identical conditions, neither RGS2 nor RGS4 bound to Gsα or G12/13α in membrane fractions as determined by Western blot analysis using specific anti-Gsα and anti-G12/13α sera (data not shown).

RGS2 and RGS4 were further tested for their capacity to interact with purified recombinant Goα and Gqα in different conformational states (Fig. 1*C*). Goα and Gqα were each incubated in the presence of appropriate agents to place them in their inactive (GDP-bound), active (GTPγS-bound), or transition state (GDP + AlF_4^--bound) conformations, and then incubated with purified histidine-tagged-RGS2, or with

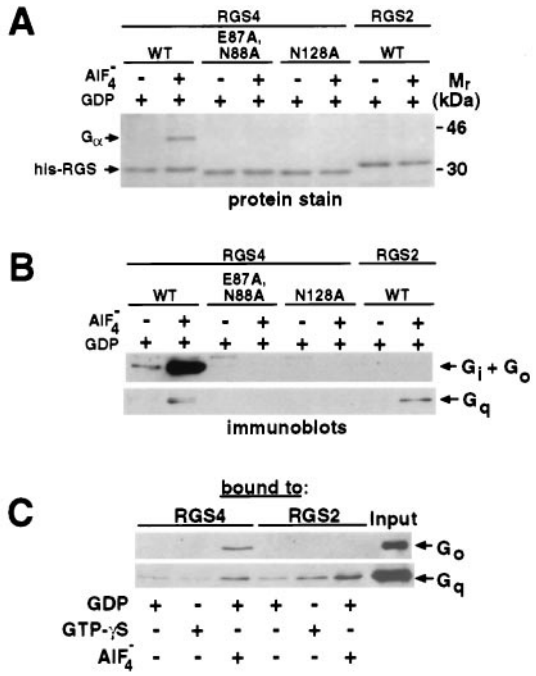

FIG. 1. Binding of RGS proteins and Gα subunits. Panels *A* and *B* show the binding of RGS2 and RGS4 to G protein α subunits in membrane fractions. The indicated wild-type or mutant forms of histidine-tagged RGS4 or wild-type histidine-tagged RGS2 were incubated with bovine brain membranes treated with GDP or GDP and AlF_4^-. Complexes containing RGS proteins were purified from detergent extracts by using Ni^{2+}-NTA chromatography. (*A*) Polypeptides bound to RGS proteins were resolved by SDS/PAGE, transferred to nitrocellulose blots and detected by staining with Ponceau S. The position where α subunits of the Gi family migrate is indicated. Mutant forms of RGS4 are smaller because they lack the first 12 amino acids of the protein, which is dispensable for GAP activity. (*B*) Identification of Gα subunits bound by RGS proteins. Nitrocellulose blots as in *A* were probed with antisera specific for the indicated G protein α subunits (and G12/13 and Gsα; data not shown) and horseradish peroxidase-coupled secondary antibodies. Enhanced chemiluminescence (Amersham) detection was used. (*C*) Interaction of RGS proteins with purified G protein α subunits in their inactive (GDP), active (GTPγS) and transition state (GDP + AlF_4^-) conformations. Binding of the indicated G protein α subunits and histidine-tagged RGS proteins was detected by isolating RGS-G protein complexes on Ni^{2+}-NTA beads and subjecting the eluted proteins to Western blot analysis. In experiments using Goα, 10% and 30%, respectively, of the input and eluted samples were analyzed. In those using Gqα, 10% of the input and eluted samples were analyzed.

wild-type or mutant forms of RGS4. RGS/Gα complexes were isolated by Ni^{2+} -NTA chromatography, subjected to SDS/PAGE and detected by Western blot analysis using specific anti-Gα sera. RGS4 bound Goα in its transition state conformation but not in its active or inactive conformations. In contrast, RGS2 did not bind detectably to Goα in any of its conformational states. RGS2 and RGS4 bound Gqα in the transition state (GDP + AlF_4^--bound) and activated (GTPγS-bound) conformation, but only poorly to the inactive (GDP-bound) protein. In all cases, RGS2 appeared to bind Gq more efficiently than RGS4. These data indicate that RGS2, but not RGS4, binds selectively to Gqα, and suggest that RGS2 may be a potent inhibitor of Gq-mediated signaling.

Comparison of the GAP Activities of RGS2 and RGS4. Measurement of Gα GTPase activity is a sensitive measure of RGS/Gα interaction that potentially could reveal interaction between RGS2 and Gi family members (23). Therefore, RGS2 and RGS4 were compared for their relative capacities to stimulate the GTPase activities of Giα1, Giα2, Giα3, Goα, Gtα, and Gsα (Fig. 2). Performing these experiments with Gqα

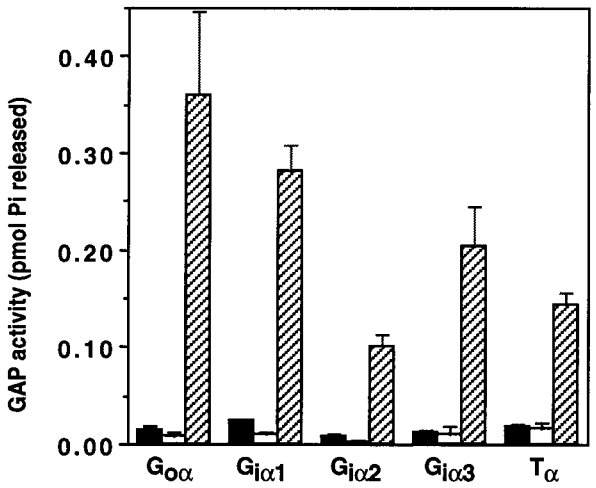

FIG. 2. Comparison of GAP activities of RGS2 and RGS4 toward various Gα subunits. The indicated G protein α subunits (100 nM final concentration) loaded with [γ-^{32}P]GTP were incubated 10 s with a buffer control (black bars), histidine-tagged RGS2 (100 nM final concentration; open bars) or histidine-tagged RGS4 (100 nM final concentration; hatched bars). The amount of ^{32}Pi released was determined by liquid scintillation spectrometry, as described in *Experimental Procedures*. The results shown are the average of two assays; SDs are indicated.

subunits is not possible due to the low affinity of Gqα for GTP (13). Consistent with previous reports (6, 7, 23), RGS4 demonstrated a clear capacity to stimulate GTP hydrolysis by all Giα family members tested. In contrast, RGS2 failed to act as a GAP for Giα family members (Fig. 2). RGS2 displayed no capacity to stimulate GTPase activity of Goα, even at a protein concentration 3,000-fold higher than is necessary to detect the effects of RGS4 (i.e., 3 μM RGS2 compared with 1 nM RGS4; data not shown). In separate experiments, RGS4 and RGS2 failed to act as GAPs for Gsα (data not shown). We did not determine whether RGS2 can act as a GAP for Gq because this requires the co-reconstitution of Gq and appropriate receptors in phospholipid vesicles, a system that is not generally available.

Inhibition of Gqα-Mediated Activation of PLCβ. RGS2 bound selectively to Gqα but failed to bind or stimulate the GTPase activity of Gi family members (Figs. 1 and 2). Therefore, we tested whether RGS2 could block Gq activation of PLCβ. We have previously demonstrated that RGS4 can block Gq-directed activation of PLC in NG-108 cell membranes, and Gqα-directed activation of PLCβ1 when reconstituted as purified proteins with phospholipid vesicles (9). These assays were used to compare the relative capacities of RGS2 and RGS4 to block Gq activation of PLC. The effects of RGS2 on Gq function in cell membranes were tested first (Fig. 3). Membranes were prepared from NG-108 cells and endogenous Gq/11α was activated with GTPγS (9). RGS2 and RGS4 at various concentrations were mixed with activated cell membranes and [^{3}H]phosphatidylinositol 4,5-bisphosphate-containing phospholipid vesicles. Membrane samples then were tested for [^{3}H]$InsP_3$ accumulation, a measure of PLC activity (9, 13). RGS2 and RGS4 both completely blocked PLC activity in a concentration dependent manner (Fig. 3). The concentration of RGS protein required to observe half maximal inhibition ($K_{0.5}$) was ≈30 nM and 300 nM for RGS2 and RGS4, respectively.

We next tested whether RGS2 could block the capacity of purified Gqα to activate purified PLCβ1 in a reconstituted system. Purified recombinant Gqα was activated with GTPγS and mixed with various concentrations of RGS2 or RGS4. These samples were added to phospholipid vesicles containing [^{3}H]phosphatidylinositol 4,5-bisphosphate and purified

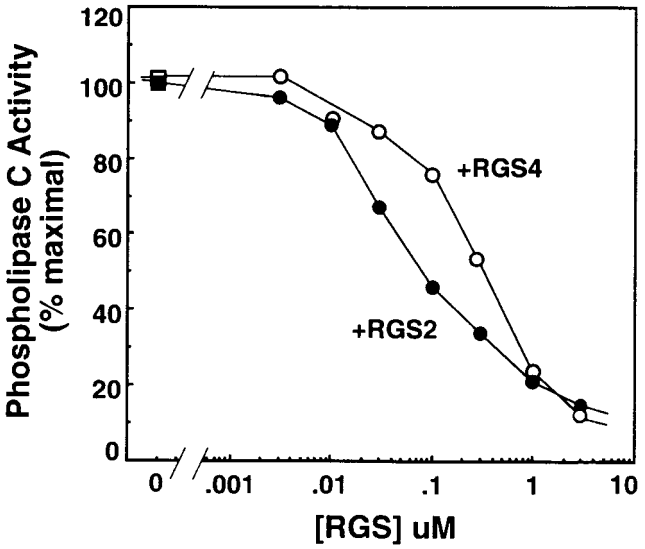

FIG. 3. Effects of RGS2 and RGS4 on GTPγS-activated synthesis of inositol 1,4,5-trisphosphate ($InsP_3$) by NG-108 cell membranes. NG-108 cell membranes (6.5 μg) were incubated as described in *Experimental Procedures* for 30 min at 30°C with 100 μM GTPγS (□, ■) or GTPγS and various concentrations of RGS2 (●) or RGS4 (○). Synthesis of [^{3}H]$InsP_3$ was measured and [^{3}H]$InsP_3$ accumulation in the absence of NG-108 membranes (blank = 118 pmol) was subtracted from each value. Values are expressed as a percentage of total [^{3}H]$InsP_3$ accumulated over 30 min at 30°C in the presence of GTPγS and absence of RGS proteins (100% = 387 pmol/assay and 424 pmol/assay for RGS4 and RGS2, respectively; basal unstimulated PLC activity was 78 pmol/assay). The data presented are the average of duplicate values and are representative of two independent experiments, each with similar results.

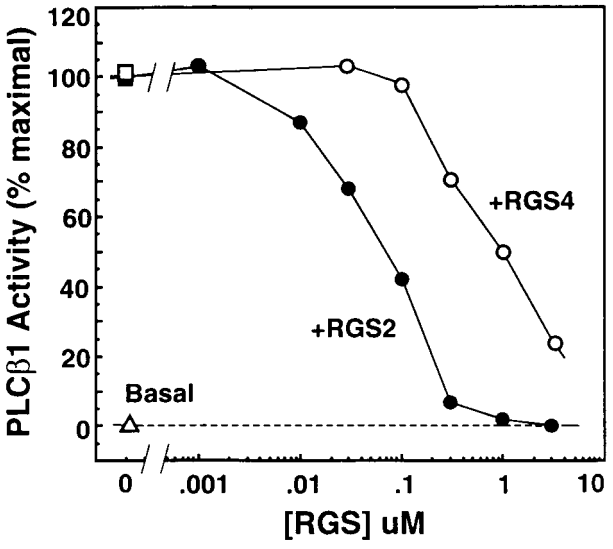

FIG. 4. Inhibition of Gqα-mediated PLC activation by RGS2 and RGS4. The effects of RGS2 or RGS4 on the activation of purified PLCβ1 by activated (GTPγS-bound) Gqα. Purified recombinant Gqα was incubated with 1 mM GTPγS for 1 hr at 30°C. Activated Gqα (1 nM final concentration) was mixed with purified recombinant PLCβ1 (1 ng) and [^{3}H]phosphatidyl inositol 4,5-bisphosphate-containing phospholipid vesicles in the absence (□, ■) or presence of various concentrations of RGS2 (●) or RGS4 (○). Synthesis of [^{3}H]$InsP_3$ was measured and basal unstimulated PLCβ1 activity (170 pmol·min·ng PLC, △) was subtracted from each value. Blank values, i.e., [^{3}H]$InsP_3$ accumulation in the absence of PLCβ1 were 155 pmol/min per assay. Values are expressed as a percentage of the total [^{3}H]$InsP_3$ accumulated over 20 min at 30°C in the presence of GTPγS-activated Gqα and the absence of RGS proteins (100% = 716 pmol/min/ng PLC and 571 pmol/min/ng PLC for the experiments involving RGS4 and RGS2, respectively). The data presented are averages of duplicate values and representative of three independent experiments, each with similar results.

PLCβ1. RGS2 and RGS4 blocked Gq activation of PLCβ1 in a concentration dependent manner (Fig. 4). RGS2 was 10- to 30-fold more potent at inhibiting Gq function than was RGS4, because the $K_{0.5}$ values were 30 nM and 1 μM for RGS2 and RGS4, respectively.

DISCUSSION

The results presented here highlight a clear physiological role for RGS2, that of a selective and potent regulator of Gq function. We reported previously that RGS4 and GAIP regulate Gq and Gi function *in vitro* (9). RGS4 was much more potent at blocking Gq function than was GAIP, and, of these two, only RGS4 blocked Gq signaling when expressed in intact cells (14, 15). These observations suggest that RGS4 but not GAIP is a physiological regulator of Gq signaling. RGS2 is now the second RGS protein clearly demonstrated to regulate Gα subunits involved in inositol lipid signaling, and it is the first family member that selectively regulates the function of Gq, but not those of Gi. RGS2 is 10-fold more potent at inhibiting Gq function than is RGS4 in reconstitution assays. The physiological significance of this is currently unclear, although it predicts that RGS2 may be a more effective regulator of Gq function in native systems. Whether RGS2 and RGS4 also regulate the functions of other Gq family members (Gqα, G11α, G14α, G15α, and G16α) remains uncertain and is currently under investigation.

Of the 20 or more mammalian RGS isoforms identified thus far, only a few have been characterized for their G protein interactions. Our findings with RGS2 raise the possibility that other poorly characterized RGS proteins may selectively regulate Gq function or signaling by other Gα subunits. To date, no RGS proteins have been shown to interact with members of the Gs or G12 family, but given the large number of RGS family members, it seems likely that some will be found that regulate these G proteins and their signaling pathways.

RGS4 is the best understood RGS protein, and information gained from the recently solved crystal structure of the RGS4-Giα1 complex (24) confirms conclusions from earlier biochemical studies indicating that RGS4 can act both as a GAP and as an effector antagonist to block G protein function. Our results indicate that RGS2 apparently acts as an effector antagonist to occlude sites on GTPγS-Gqα that interact with PLCβ. We suspect that RGS2 also can act as a GAP for Gqα, although our ability to test this idea directly has been limited by the unavailability of purified receptor protein which is necessary to load GTP onto Gqα (9, 25). Indirect evidence that RGS2 may function as a GAP is provided by our studies showing that RGS2 binds preferentially to the transition state (GDP + AlF_4^--bound) conformation of Gqα. RGS4 binds with high affinity to the analogous transition state conformation of Giα1, and structural and mutational data suggest that RGS4 stabilization of specific residues in the so called switch I, II, and III regions of Giα1 account for the capacity of RGS4 to act as a GAP (ref. 21; S. Srinivasa, N.W., and K.J.B.; unpublished results).

RGS2 is the only RGS protein studied to date that exhibits no capacity to interact with Giα family members. How this specificity is achieved is currently unclear since the overall amino acid sequences of RGS2 and other RGS proteins that interact with Giα family members are not strikingly different. However, clues may be provided by the RGS4-Giα1 crystal structure (24). Contacts between RGS4 and Giα1 appear to be limited to the three switch regions of Giα1 important for GTP binding and hydrolysis, and the 120 amino acid core domain of

RGS4 that forms a bundle of nine α-helices; this "RGS box" is conserved among all RGS proteins. The switch I region of Gα is particularly important for these interactions. Seven residues within RGS4 interact with switch I residues of Giα1 (most notably Thr-182) and five of these seven are invariant among RGS proteins (21). The remaining two residues, Ser85 and Asp163 of RGS4, are replaced by cysteine and asparagine residues, respectively, in RGS2. It will be of interest to determine whether switching these amino acids in RGS4 and RGS2 alter their selectivities for Gα interactions. Whether other regions in RGS2 contribute to its capacity to distinguish between Giα family members and Gq remains to be determined.

The fact that RGS2 is a selective regulator of Gq function while RGS4 is not raises important questions regarding the relative biological roles for RGS2 and RGS4 in native systems. One issue centers on whether activation of Gq-linked signaling pathways regulate the cellular levels of one or more RGS proteins, in turn, to regulate a second temporally removed Gq-mediated signaling event. This in fact may be the case for RGS2 since its mRNA is specifically induced by agents that increase cellular levels of inositol phosphates and intracellular calcium in human monocytes (20). We cannot rule out the possibility that other (non-Gq-linked) signaling pathways also act to regulate cellular RGS2 levels to serve a central role in heterologous desensitization of Gq signaling. The cellular levels of RGS4 also may be regulated by extracellular signals, although its specific roles in desensitization are necessarily more complex because it regulates both Gq- and Gi-linked signaling pathways. Other determinants are also certainly involved. RGS4 is lipid modified by palmitate at cysteine residues in an N-terminal domain that is important for plasma membrane targeting of RGS4 (S. Srinivasa, L. Friedman, K.J.B., and M.E.L.; unpublished results). Furthermore, RGS4 is expressed primarily in brain (12) whereas RGS2 is expressed broadly in many tissues (19). Given this information, it is likely that such factors as subcellular localization, tissue and cell-type distribution, posttranslational modification and temporal expression conspire to dictate which RGS proteins regulate which G protein signaling pathways in a given cell.

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