Polarity Exchange at the Interface of Regulators of G Protein Signaling with G Protein α-Subunits*

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RGS proteins are GTPase-activating proteins (GAPs) for G protein α-subunits. This GAP activity is mediated by the interaction of conserved residues on regulator of G protein signaling (RGS) proteins and Go-subunits. We mutated the important contact sites Glu-89, Asn-90, and Asn-130 in RGS16 to lysine, aspartate, and alanine, respectively. The interaction of RGS16 and its mutants with Goi and Goi1 was studied. The GAP activities of RGS16N90D and RGS16N130A were strongly attenuated. RGS16E89K increased GTP hydrolysis of Goi1, with the inactive GDP-bound form, which then reassembles with the Gβγ dimer, and thus terminates signaling. Recently, a novel superfamily of GTPase-activating proteins (GAPs) for Go-subunits, termed “regulators of G protein signaling” (RGS) proteins, with at least 21 different mammalian members, has been discovered (for review see Refs. 4–6). Besides their obvious role in regulation of signal strength and duration, RGS proteins have been shown to be involved in desensitization processes. In vitro reconstitution experiments, a variety of RGS proteins exerts GAP activity for members of the Goi and Goi1 subfamilies with a limited degree of specificity (for review see Ref. 6). On the molecular level, this lack of specificity is not very surprising. When the structure of the RGS4-Goi1 complex was solved by crystallization and X-ray diffraction (7), it became evident that interaction of these molecules involved the Goi1 amino acids Thr-182, Glu-204, Glu-207, Lys-210, and Lys-215. This led to the concept that mutation of conserved lysine residue to glutamate in Goi and Goi1 family members renders these proteins insensitive to wild type RGS proteins. Nevertheless, they are sensitive to glutamate to lysine mutants of RGS proteins. Such mutant pairs will be helpful tools in analyzing Go-Gs RGS specificities in living cells.

Many hormones, neurotransmitters, and sensory stimuli use surface receptors coupled to heterotrimeric (Goβγ) guanine nucleotide-binding proteins (G proteins)1 to transmit extracellular signals (1–3). To elicit an appropriate cellular response the strength and duration of intracellular signals must be tightly regulated. The duration of G protein activation itself is controlled by the intrinsic GTPase activity of Go-subunits. GTP hydrolysis converts the active GTP-bound Go-subunit to the inactive GDP-bound form, which then reassembles with the Gβγ dimer, and thus terminates signaling. Recently, a novel superfamily of GTPase-activating proteins (GAPs) for Go-subunits, termed “regulators of G protein signaling” (RGS) proteins, with at least 21 different mammalian members, has been discovered (for review see Refs. 4–6). Besides their obvious role in regulation of signal strength and duration, RGS proteins have been shown to be involved in desensitization processes.

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‡‡ The abbreviations used are: G protein, heterotrimeric (Goβγ) guanine nucleotide-binding protein (G proteins)1 to transmit extracellular signals (1–3). To elicit an appropriate cellular response the strength and duration of intracellular signals must be tightly regulated. The duration of G protein activation itself is controlled by the intrinsic GTPase activity of Go-subunits. GTP hydrolysis converts the active GTP-bound Go-subunit to the inactive GDP-bound form, which then reassembles with the Gβγ dimer, and thus terminates signaling. Recently, a novel superfamily of GTPase-activating proteins (GAPs) for Go-subunits, termed “regulators of G protein signaling” (RGS) proteins, with at least 21 different mammalian members, has been discovered (for review see Refs. 4–6). Besides their obvious role in regulation of signal strength and duration, RGS proteins have been shown to be involved in desensitization processes.

EXPERIMENTAL PROCEDURES

Animals—Female Wistar rats were obtained from a colony bred and maintained at the animal house of the University Hospital Eppendorf. Myometria were dissected from the rats after anesthesia with halothane followed by cervical dislocation. All experimental procedures

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were carried out according to the animal welfare guidelines of the University Hospital Eppendorf.

**Mutation of cDNAs—** Mutations of mouse RGS16 cDNA were performed by polymerase chain reaction using pETi15B-His_i1-RGS16 (9) as template. Mutagenic primers were as follows: primer 1, 5′-CTTGGGGGCTGATCTCTGGTT-3′, mutant primer 2, 5′-CCCCATCAGGCATCAATC-3′, mutant primer 3, 5′-GAGGTTGCGGCCTGACATTC-3′, mutant primer 4, 5′-GATATGCAGAGAGGATCTTCTTGTGAG for the double mutant Gαq_i1E207Q/K210E, and Gαq_i1E89K/G120A for Gαq_i1E89K/K210E.

**Preparation of Uterine Smooth Muscle Cells and Electrophysiological Recording of Large Conducance Ca2+—activated K+ (BKCa) Channel Activity—** Rat myometrial smooth muscle cells were isolated from the uterus as described before (19). BKCa activity was recorded using the whole-cell patch configuration. Recombinant proteins were dialyzed into the cells via the patch pipette. All experimental procedures were performed essentially as reported before (19).

**Transfection of COS-7 Cells, Determination of Recombinant Protein Expression, and Phospholipase C Activity—** COS-7 cells were seeded at a density of 10⁵ cells per well in 12-well tissue culture plates 1 day before transfection. The amount of DNA used for transfection with pCis-Gαq_i1-RGS16 and pCis-Gαq_i1-RGS16/K210E was 0.2 μg, for pCR3-Gαq and pCR3-GαqS4E57K 0.8 μg. A plasmid expressing the β-galactosidase gene under the control of the cytomegalovirus promoter (pCis-LacZ) was cotransfected to keep the total amount of DNA in each transfection constant at 1 μg; 0.5 μl of Opti-MEM (Life Technologies, Inc.) was added to each well. Twelve hours later, the medium was replaced with 1 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Twenty-four hours post-transfection, cells were labeled with 10 μCi of myo-2-¹⁵N-hinositol (NEF Life Science Products) per ml in 0.5 ml of isonitric-free medium containing 10% dialyzed fetal bovine serum. Forty eight hours post-transfection, the levels of [¹⁵N]hinositol phosphate were determined as described (20, 21). For detection of recombinant protein expression, cells were kept for the same time in Dulbecco’s modified Eagle’s medium after replacing the transfection medium and then harvested in sample buffer (21). Proteins were separated by PAGE (15% polyacrylamide in the resolving gel) and electrophoretically transferred onto nitrocellulose membranes. Gαq11 proteins were visualized with an anti-Gαq11 anti-serum raised against a C-terminal epitope (Calbiochem) and the ECL system (Amersham Pharmacia Biotech).

**RESULTS**

**GAP Activity of RGS16 and Its Mutants for Gαq—** The crystal structure of the Gα12-RGS4 complex (7) indicated that side chains of several amino acids are required for the interaction of RGS proteins with Gα-subunits of the Gα12 and Gαq subfamily members. We therefore mutated three amino acids corresponding to these important contact sites (Fig. 1). Glu-89, Asn-90, and Asn-130 in RGS16 to lysine, aspartate, and alanine, respectively. Wild type RGS16 and the mutants were expressed in E. coli and purified as described before (9). The ability of recombinant RGS16 mutants to act as GAP was studied in the presence of a nonturnover GTPase assay for Gα that was originally used to characterize RGS16 GAP activity (9, 17). A maximal effective concentration of RGS16 (1 μM) increased GTP hydrolysis by Gαq 7–8-fold (Fig. 2A). In contrast, the GAP activity of RGS16N130A (1 μM) was reduced by 90–95%, RGS16E89K and RGS16N90D did not exhibit any significant GAP activity. Single turnover GTPase of Gαq, however, revealed a different
RGS16N90D were not evident on this GTPase (see Fig. 4) and binding of the GTP analog (E89K, 1 \mu M) stimulated GTP hydrolysis by about 7-fold. RGS16E89K (1 \mu M) stimulated GTP hydrolysis by about 3-fold (Fig. 3). The half-maximal and maximal increase in Pi release was observed at 20 and 100 nM RGS16, respectively. The rate constant for GTPase activity were 7.9 \pm 1 \times 10^{-3} s^{-1} and 4.6 \pm 0.3 \times 10^{-3} s^{-1} for Goa1 and Goa1K210E, respectively. In the presence of RGS16 and RGS16E89K, the rate constant for Goa1 increased to 94.5 \pm 11 \times 10^{-3} s^{-1} and 16.2 \pm 0.1 \times 10^{-3} s^{-1}, respectively. The rate constant for Goa1K210E increased to 42.7 \pm 0.4 \times 10^{-3} s^{-1} in the presence of RGS16E89K, indicating that under the experimental conditions used, maximal acceleration of GTP hydrolysis is similar (about 10-fold) for the interaction of Goa1 with RGS16 and of Goa1K210E with RGS16E89K, respectively.

In order to increase the apparent affinity of Goa1K210E for RGS16E89K, double mutants of Goa1 and RGS16 were studied. First, we generated the double mutant pair RGS16E89K/N90D...
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**Fig. 5.** Concentration dependence of the GAP activity of RGS16 and RGS16E89K for Goq and GoαK210E. Single turnover GTPase of 250 nM recombinant Goq (A) or GoαK210E (B) in the absence and presence of increasing concentrations of RGS16 (○) or RGS16E89K (●). Data shown are means ± S.D. of assay triplicates.

**Fig. 6.** Acceleration of P released from Goq and GoαK210E by RGS16 and RGS16E89K. P release from 250 nM recombinant Goq (A) or GoαK210E (B) was determined in the absence (□) and presence of RGS16 (○, 100 nM) or RGS16E89K (●, 10 μM) at the indicated periods. The data shown are the average of two independent experiments.

and GoqE207Q/K210E. Second, as GoqK210E apparently has a rather acidic surface consisting of Glu-207 and Glu-210, a more basic counterpart was created with the double mutant RGS16E89K/N90K. Finally, as in this mutant the side chain of Lys-90 has two more carbon atoms than that of the original asparagine, and the side chain of Glu-207 was shortened in the double mutant GoqE207D/K210E for compensation. All the Goq double mutants exhibited an unaltered rate of basal GTP hydrolysis and GTPγS binding (data not shown). They failed, however, to be stimulated in their rate of GTP hydrolysis by RGS16 or any of its mutants. Also the double mutants of RGS16 did not increase the GTPase activity of Goq, GoqE207Q, or GoqK210E (Table I).

Influence of RGS16, RGS16E89K, Goq, and GoαK210E on BKCa Channel Activity in Rat Uterine Smooth Muscle Cells—If RGS16E89K and GoαK210E bare the potential to serve as tools in analyzing Go-RGS protein interaction, the differences between the mutants and their wild type counterparts observed in the in vitro GAP assay have to cause distinguishable differences in the modulation of signaling pathways by the mutants and wild type proteins on the background of a living cell. To test this hypothesis we chose the model of the BKCa channel regulation via the adenylyl cyclase (AC)-protein kinase A (PKA) pathway in uterine smooth muscles cells. As shown before (19), under the experimental conditions chosen, the BKCa channel activity is a very sensitive probe to follow AC-PKA activation and even more important, defined concentrations of purified proteins can be dialyzed into the cell via the patch pipette. Therefore, this method allows us to study the influence of these proteins on regulatory components of the AC activity in detail. As shown in Fig. 7, stimulation of the Gq-coupled ß-adrenoreceptors on these cells by isoproterenol (10 μM) caused a pronounced decrease of the outward current through BKCa channels (Iout) by about 50%. Co-stimulation of the Gq-coupled qβ-adrenoreceptors by the selective agonist clonidine (10 μM) antagonized the effect of isoproterenol by partially restoring Iout to about 80% of the drug-free control. After dialysis of 1 μM RGS16 into the cells, the Gq-mediated effect of clonidine, however, was totally abolished. In contrast, RGS16E89K, even when used in a concentration of 10 μM, was without effect on the isoproterenol- and clonidine-evoked signals.

As the inhibitory effect of qβ-adrenoreceptors on AC activity is mediated by Goq-subunits (19), this inhibition should be mimicked by dialyzing recombinant Goq into the cells. As shown in Fig. 8, 1 μM Goq completely antagonized the inhibition of Iout caused by isoproterenol, whereas the currents in the presence of 0.1 μM Goq were virtually unaltered. Interestingly, GoqK210E was about 10-fold more potent than wild type Goq. At 0.1 μM GoqK210E the inhibitory effect of isoproterenol was completely blunted. In contrast to its inability to regulate endogenous G proteins (see Fig. 8), RGS16E89K (10 μM) completely reversed the effect of 0.1 μM GoqK210E when both proteins were applied simultaneously to the cells.

**Interaction of GoαK210E with Gβγ**—The difference in potency of Goq and GoqK210E to inhibit AC activity in the uterine smooth muscle cells might be explained by an impaired ability of the mutant to form heterotrimers with Gβγ. To exclude this possibility we tested heterotrimer formation of GDP-ligated Goq and GoqK210E (2.5 μM) with an equimolar concentration of Gβγ. As shown in Fig. 9, Goq and GoqK210E similarly trapped Gβγ in an apparent 1:1 proportion to Ni2+-NTA beads via their internal His6 tag. Gβγ alone did not bind to the matrix. Thus, the ability of heterotrimer formation is apparently not impaired by the Lys to Glu mutation.

Interaction of RGS4 and RGS4E87K with GoqR183C and GoqR183C/K215E, Respectively—To study whether the concept of creating an interacting mutant pair can be extended to other RGS proteins and members of the Goα subfamily, we introduced mutations analogous to RGS16E89K and GoqK210E in RGS4 (RGS4E87K) and Goq (GoqK215E, see Fig. 1). As template for the Goq mutation we used the cDNA of the GoqR183C mutant. This mutant has been shown to be GTPase-deficient by itself, whereas it regains GTPase activity in the presence of RGS4 (22). In addition, it can be used in transfection assays to stimulate directly phospholipase C (PLC) activity in COS-7 cells (23).

First we checked the expression of GoqR11 proteins in transfected COS-7 cells by immunoblotting. Transfection with the
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TABLE I
Maximal GAP activities of RGS16 and its mutants for Gaq and its mutants

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<tr>
<td>RGS16</td>
<td>3.17 ± 0.34</td>
<td>2.20 ± 0.55</td>
<td>1.10 ± 0.09</td>
<td>0.97 ± 0.10</td>
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<td>RGS16E89K</td>
<td>2.86 ± 0.88</td>
<td>1.10 ± 0.10</td>
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<td>RGS16N90D</td>
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<td>1.07 ± 0.06</td>
<td>1.43 ± 0.20</td>
<td>0.86 ± 0.04</td>
<td>1.21 ± 0.21</td>
</tr>
<tr>
<td>RGS16E89K/N90D</td>
<td>0.96 ± 0.13</td>
<td>1.08 ± 0.20</td>
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<td>RGS16E89K/N90K</td>
<td>1.1 ± 0.09</td>
<td>1.32 ± 0.32</td>
<td>1.13 ± 0.21</td>
<td>1.09 ± 0.20</td>
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a p < 0.001 versus basal activity.
b p < 0.05 versus basal activity.

Fig. 7. Influence of RGS16 and RGS16E89K on α-adrenoceptor-mediated regulation of BKCa channel activity in rat uterine smooth muscle cells. Currents were elicited in rat uterine myocytes every 10 s by 200-ms depolarizing pulses from −20 to +80 mV in the presence of 0.3 μM intracellular Ca2+. Cells were first dialyzed via the patch pipette for 20 min with intracellular pipette solution alone (Control) or the indicated concentrations of RGS16 (WT) and RGS16E89K (E89K), and thereafter superfused with 10 μM isoprotrenenol (2), open bars), followed by application of 10 μM clonidine (3), closed bars). A, a representative original recording for each condition is presented. B, average results from 5 to 7 cells obtained from different rats are shown. Current densities were used to calculate the percentage values. Data are expressed as means ± S.E. Statistical significance was determined by Student’s t test for paired observations. ***, p < 0.01; *, p < 0.05; versus isoprotrenenol.

Fig. 8. Influence of Gaq and Gaq,K210E on BKCa channel activity in rat uterine smooth muscle cells. Currents were elicited in rat uterine myocytes every 10 s by 200-ms depolarizing pulses from −20 to +80 mV in the presence of 0.3 μM intracellular Ca2+. Cells were first dialyzed via the patch pipette for 20 min with intracellular pipette solution alone (Control) or the indicated concentrations of Gaq,WT, Gaq,K210E, and RGS16E89K. Thereafter, cells were superfused without (1, open bars) and with 10 μM isoprotrenenol (2, closed bars). A, a representative original recording for each condition is presented. B, average results from 5 to 9 cells obtained from different rats are shown. Current densities are given as means ± S.E. Statistical significance was determined by Student’s t test for paired observations. ***, p < 0.01; ***p < 0.001; versus drug-free.

C DNA encoding RGS4 proteins did not alter the amount of endogenously expressed Gaq11 proteins (Fig. 10, upper panel). The cDNA coding for Gaq,R183C or Gaq,R183C/K215E clearly increased the levels of immunodetectable Gaq11 proteins under all conditions tested. Cotransfection with the plasmid for RGS4 slightly decreased the expression levels of Gaq,R183C and Gaq,R183C/K215E, whereas the plasmid for RGS4E87K was without inhibitory effect. As shown in Fig. 10 (lower panel), PLC activity in COS-7 cells increased about 2-fold when transfected with the plasmid encoding Gaq,R183C or Gaq,R183C/K215E. PLC activity decreased by 57% after transfection of an RGS4 encoding plasmid alone. RGS4E87K encoding DNA had little if any effect. Coexpression of RGS4 with Gaq,R183C totally abolished Gaq,R183C-induced PLC activity, whereas coex-pression of RGS4E87K had no effect. In contrast, Gaq,R183C/ K215E-induced PLC activity was sensitive to coexpression of RGS4E87K but not of RGS4. The moderate differences in the expression levels of Gaq,R183C and Gaq,R183C/K215E are much smaller than the alterations in PLC activity. Therefore, the large reduction in PLC activity after cotransfection of RGS4 + Gaq,R183C and RGS4E87K + Gaq,R183C/K215E indicates a negative regulation of Gaq,R183C and Gaq,R183C/K215E by RGS4 and RGS4E87K, respectively.
Drolysis. The observation that GTPase-deficient Q204L 

Gα and Gβ transfected with the mammalian expression vector pCis encoding Blue.

beads, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue. Lane 1, Gβγ was directly applied to the gel as a loading control.

FIG. 9. Interaction of Gαq and GαbgK210E with Gβγ. Gαγ (2.5 μM) was incubated without (lane 2) and with an equimolar concentration of Gαq (lane 3) or GαbgK210E (lane 4) in the presence of 10 μM GDP. Thereafter, His6-tagged Gαγ proteins were trapped to a Ni2+-NTA matrix, and beads were extensively washed as described under “Experimental Procedures.” Bound proteins were eluted from the beads, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue. Lane 1, Gβγ was set to 100%. Data in the lower panel are means ± S.D. of one representative experiment performed in triplicate.

FIG. 10. Interaction of RGS4 and RGS4E87K with Gαq-R183C and GαqR183C/K215E, respectively. COS-7 cells were transiently transfected with the mammalian expression vector pCis encoding β-galactosidase (LacZ), Gαq-R183C, or GαqR183C/K215E (open bars) or were cotransfected with these vectors plus RGS4- (cross-hatched bars) or RGS4E87K-encoding constructs (filled bars). Forty-eight hours posttransfection, inositol phosphate accumulation (lower panel) and expression of Gαqγ1 proteins (upper panel) were analyzed as described under “Experimental Procedures.” PLC activity in cells transfected only with pCis-LacZ was set to 100%. Data in the lower panel are means ± S.D. of one representative experiment performed in triplicate.

Discussion

The majority of the so far identified RGS proteins are GAPs for members of the Gαi and Gαq subfamilies with a very limited degree of specificity (6). From a structural point of view, this is not too surprising. When the complex of RGS4 with Gαi was crystallized (7), it became evident that RGS4 interacts with the important switch regions, switch I, II, and III in Gαi. The conformations of these switch regions are most sensitive to the binding of GDP or GTP to the Gαi-subunit. Residues in switch I and II are directly involved in binding and hydrolysis of GTP. In the same region, residues in switch I (Thr-182) and switch II (Gln-204, Glu-207, and Lys-210) of Gαi form hydrogen bonds with residues (Tyr-84, Glu-87, Asn-88, and Asn-128) of RGS4. All members of the Gαi and Gαq subfamilies on the one hand and a majority of RGS proteins on the other hand are highly conserved at these important contact sites (see Fig. 1). Tesmer et al. (7) also suggested that the direct interaction of Aα-128 in RGS4 with Gln-204 in Gαi is of major importance to orient Gln-204 of Gαi in an optimal conformation to the attacking water molecule and the γ-phosphate of GTP during GTP hydrolysis. The observation that GTPase-deficient Q204L mutants of Gαi family members are able to bind to RGS proteins (24), but do not regain GTPase function in the presence of RGS4 (18), further support this hypothesis. Our data obtained by mutation of the analogous Asn-130 in RGS16 (see Fig. 1) to alanine indicate that this residue is indeed critical for their GAP function. Although the GAP activity of RGS16N130A for Gαi and Gαq was not completely abolished, its efficacy was reduced by 90–95%. Mutation of Asn-128 in RGS4 to alanine was reported to abolish its GAP activity (25). In addition, RGS proteins, like GAIP, with a serine at the position corresponding to Asn-128 in RGS4, are less potent than RGS4 but still exhibit GAP activity for several Gαi-subunits (26–28). Recent work by others (29, 30) indicates that this residue is very important for binding of RGS proteins to Gαi-subunits and variably modulates the maximal rates of GTP hydrolysis. Thus, the extent of loss in GAP activity by alteration of this important asparagine differs between certain pairs of RGS proteins and Gαi-subunits.

As shown herein, mutation of Asn-90 to Asp abolished GAP activity of RGS16 for Gαi and Gαq. Similarly, mutation of the analogous Asn-88 to Ser in RGS4 abolished interaction with Gαq (31), indicating that this conserved residue is also essential for the interaction of RGS proteins with Gαq family members. In contrast, mutation of Glu-89 to Lys did not abolish interaction of RGS16 with Gαi or Gαq. It has been shown before that the EC50 of the GAP activity is a good estimate for the affinity of RGS proteins to Gαi-subunits (17). Therefore, the high EC50 of RGS16E87K represents a markedly (about 100-fold) reduced affinity for Gαq family members. In addition, Glu-89 of RGS16 is most likely to interact with Lys-210 in Gαi. When this residue in Gαi was changed to glutamate, this mutant was insensitive to RGS16. The GTP hydrolysis by GαqK210E could, however, still be stimulated by RGS16E89K to a similar extent as observed for the RGS16-Gαq interaction. Taken together, these data indicate that the interaction of this highly conserved glutamate in RGS proteins with the equally well conserved lysine in Gαq and Gαq family members is important for high affinity binding but is apparently not directly involved in the acceleration of GTP hydrolysis.

For many RGS proteins it is not known if they gain specificity for the different members of the Gαi and Gαq subfamilies. Thus, the insensitivity of GαqK210E to RGS16 and its efficient interaction with RGS16E89K is of particular interest. To establish the significance between wild type and mutant proteins observed in the in vitro GAP assay, we tested all proteins in vivo for differential regulation of endogenous signaling pathways on a cellular background. In rat uterine smooth muscle cells, we monitored the α2-adrenoreceptor Gαi-mediated inhibition of AC by the PKA-mediated regulation of BKCa channel activity (19). In contrast, mutation of Glu-89 to Lys did not significantly alter the α2-adrenoreceptor effect. Both, recombinant Gαq and GαqK210E mimicked the effect of the endogenous Gαi and inhibited AC activity to a similar extent. Their potency, however, was significantly (about 10-fold) different. The maximal inhibition by Gαq and GαqK210E was observed at 1 and 0.1 μM, respectively. As both proteins exhibited a similar basal GTPase rate (see Figs. 4 and 5), this difference could be explained by their different susceptibility to the endogenous RGS proteins, of which we detected several in the uterine smooth muscle cells by RNase protection assay and immunoblot (data not shown). Whereas the half-life of Gαi-GTP was shortened by RGS proteins, that of GαqK210E-GTP should not. Therefore, maximally inhibitory concentrations of of GTP-ligated αi-subunit were obtained at a lower concentration of the mutant protein compared with wild type Gαi. This interpretation was further corroborated by the ability of RGS16E89K to reverse
Gaq K210E effects in the uterine smooth muscle cell (see Fig. 8). Together with the unimpaired ability of Gaq K210E to form a heterotrimer with Gβγ (see Fig. 9), these data argue against other possible explanations for the increased potency of Gaq K210E like impaired heterotrimer formation or increased affinity of the mutant for AC isoforms. In summary, our data suggest that in a living cell the action of Gaq K210E is independent of endogenously expressed RGS proteins, and RGS16 E89K is a extremely poor negative regulator of endogenous Gα subunits. In addition, corresponding mutants of RGS4 (Glu-87 to Lys) and Gaq (Lys-215 to Glu, see Fig. 1) exhibited similar properties when the stimulation of PLC activity by a GTPase-deficient Gaq mutant (Gaq R183C) in transfected COS-7 cells was studied. The activity of overexpressed Gaq R183C was negatively regulated by RGS4 but not by RGS4E87K, whereas Gaq R183C/K215E was sensitive to expression of RGS4E87K but not to RGS4. In contrast to RGS4E87K, expression of RGS4 alone inhibited PLC activity by 57%. This inhibition is most likely due to the interaction of RGS4 with endogenously expressed Gaq family members and thus is in line with the other data. As outlined before, the inhibition of PLC activity should reflect the interaction and thus inactivation of Gaq and Gα215E by RGS4 and RGS4E87K, respectively. Together with data obtained with RGS16 E89K and Gaq K210E in vitro and in vivo, they therefore implicate that by mutation of the highly conserved glutamate to lysine in RGS proteins and lysine to glutamate in Gaq subunits a pair interacting mutants can be created.

Two recent studies (32, 33) indicate that certain RGS proteins contain motifs in the N terminus that contribute to specificity in regulation of distinct signaling pathways. In rat pancreatic acinar cells, the Ca2+ release evoked by Gaq-coupled receptors was differentially regulated by RGS4. The m3 muscarinic receptor-induced Ca2+ release was about 3-fold and 10-fold more sensitive to RGS4 than that induced by bombesin and cholecystokinin receptors, respectively. Moreover, the discrimination between m3 and cholecystokinin receptor-evoked signals differed between RGS family members. Whereas RGS2 was equally potent in inhibiting both signals, RGS1 was about 1000-fold more potent in inhibiting m3 receptor signaling (33). Experiments performed with knock-out mice lacking different members of the Gaq11 subfamily indicate that RGS proteins interact equally well with all members of this subfamily. These data question that this specific subset of RGS proteins does discriminate between members of the Gaq and Gaq subfamilies. Nevertheless, it is frequently encountered that Gaq-coupled receptors coactivate Gαi subfamily members (34). This has also been demonstrated for the m3 receptor (35). It is not clear to date whether RGS proteins are involved in post-receptor signal sorting. We have shown herein for Gaq and Gaq that a lysine to glutamate mutation renders the Gα subunit insensitive to wild type (in vitro) and endogenously (in vivo) expressed RGS proteins. On the other hand, glutamate to lysine mutants of interacting RGS proteins were able to restore the original signaling. Our data implicate that the introduced mutations in RGS proteins and Gaq subunits do not affect other domains, e.g. the interaction sites with receptor, effectors, or other proteins. Thus, the mutated proteins are selectively uncoupled from endogenous signal transduction at the level of RGS-Gα subunit interaction but are otherwise functionally intact. Expression of a single mutant (RGS protein or Gaq subunit) might therefore create a phenotype that can be rescued by the corresponding mutant of a specific RGS-Ga couple. Therefore, the interacting mutant pairs are supposed to be a helpful tool to analyze RGS-Ga subunit interaction in living cells or even transgenic animals.

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