Regulator of G Protein Signaling Proteins: Novel Multifunctional Drug Targets

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ABSTRACT

G protein-coupled receptors (GPCRs) play a major role in signal transduction and are targets of many therapeutic drugs. The regulator of G protein signaling (RGS) proteins form a recently identified protein family, and they strongly modulate the activity of G proteins. Their best known function is to inhibit G protein signaling by accelerating GTP hydrolysis [GTPase activating protein (GAP)] thus turning off G protein signals. RGS proteins also possess non-GAP functions, through both their RGS domains and various non-RGS domains and motifs (e.g., GGL, DEP, DH/PH, PDZ domains and a cysteine string motif). They are a highly diverse protein family, have unique tissue distributions, are strongly regulated by signal transduction events, and will likely play diverse functional roles in living cells. Thus they represent intriguing, novel pharmacological/therapeutic targets. Drugs targeting RGS proteins can be divided into five groups: 1) potentiators of endogenous agonist function, 2) potentiators/desensitization blockers of exogenous GPCR agonists, 3) specificity enhancers of exogenous agonists, 4) antagonists of effector signaling by an RGS protein, and 5) RGS agonists. In addition, a novel subsite distinction within the RGS domain has been proposed with significant functional implications and defined herein as “A-site” and “B-site”. Therefore, RGS proteins should provide exciting new opportunities for drug development.

G protein-coupled receptors (GPCRs) play a major role in signal transduction and are the targets of a large number of therapeutic drugs. Just as our understanding of receptor, G protein, and effector function seemed nearly complete, a new kid appeared on the scene injecting fresh life into the field. The regulator of G protein signaling (RGS) proteins modulate the activity of G proteins in vitro, and evidence is beginning to emerge on their role in vivo as well. The regulator of G protein signaling (RGS) proteins modulate the activity of G proteins in vitro, and evidence is beginning to emerge on their role in vivo as well. Their best known function is to inhibit G protein signaling by accelerating GTP hydrolysis thus turning off G protein signals (Berman et al., 1996a). They are a highly diverse protein family, have unique tissue distributions, and are strongly regulated by signal transduction events. Also, evidence is emerging that besides G protein inhibition, they can enhance G protein activation, serve as effectors, and act as scaffold proteins to gather receptors, G proteins, effectors, and other regulatory molecules together.

There have been several excellent reviews on RGS proteins recently (Hepler, 1999; Siderovski et al., 1999; De Vries et al., 2000; Ross and Wilkie, 2000), so we will focus on known or predicted physiological functions of RGS proteins and on concepts related to RGS proteins as potential drug targets (see also Jones et al., 2000 and Dohlman, 2001).

A Brief History of Regulators of G Protein Signaling

The RGS proteins were discovered in genetic studies of GPCR signaling pathways in model organisms (Dohlman and Thorner, 1997). The scope and significance of RGS proteins were recognized in 1996 when ~20 mammalian members of the RGS protein family were identified based on sequence comparisons.
homologies with a conserved 120-amino acid domain in the original yeast and worm RGS proteins, Sat2 and EGL-10, respectively (Druey et al., 1996; Koelle and Horvitz, 1996; Siderovski et al., 1996).

Later in 1996, several groups showed that RGS proteins were GAPs (for GTPase accelerating protein) (Berman et al., 1996a). The crystal structure of a Ga1-RGS4 suggested a mechanism for the GAP activity: stabilization of the transition state conformation of Ga (Berman et al., 1996b). The GAP activity explains RGS-mediated inhibition of G protein signaling. It also explains the paradox that some signals, visual responses and cardiac potassium channels (Szabo and Otero, 1989; Arshavsky et al., 1994), turn off much faster than expected given the slow hydrolysis of GTP by purified Ga subunits.

**More Than Just Ga GAPs**

Recently, there has been a paradigm shift in thinking about RGS proteins (Hepler, 1999; Siderovski et al., 1999). In addition to GAP activity, RGS proteins also: 1) directly antagonize Ga effectors (Hepler et al., 1997; Tesmer et al., 1997), 2) bind Gβγ (Snow et al., 1998b), 3) potentially target protein kinase A and receptor kinases since AKAP contains an RGS-like domain and is a predicted RGS family member (Koch et al., 1993; Huang et al., 1997), 4) scaffold Wnt signaling proteins (reviewed in Kikuchi, 1999), 5) are Ga13 effectors activating Rho (Hart et al., 1998; Kozasa et al., 1998), and 6) enhance receptor-G protein coupling (see below). Thus, we should think of RGS domains as modular, regulatable, Ga subunit recognition domains along the lines of SH2, SH3, or PDZ domains. As SH2 domains only bind tyrosine-phosphorylated peptides, RGS binding to Ga depends on the Ga functional state. For example, RGS4 binds only to the (AlF$_4^-$-bound) transition state of Ga1 subunits, while RGS2 binds both transition state and active (GTPγS-bound) Gaα, and neither of them binds to the resting state of Ga (Berman et al., 1996b; Heximer et al., 1997). This produces state-dependent recruitment of the RGS protein to the vicinity of Ga subunits.

Effector antagonism uses the RGS domain (Hepler et al., 1997; Tesmer et al., 1997), but most other non-GAP functions involve functional domains in N- and C-terminal extensions. The wide array of domains found in RGS proteins has been reviewed recently (Hepler, 1999; Siderovski et al., 1999; De Vries et al., 2000) and includes GGL (G protein γ-subunit-like), DEP (disheveled, egl-10, pleckstrin), DH/PH (Dbl/pleckstrin homology) and PDZ (PSD-95, disc-large, and ZO-1 homology) domains. These non-RGS components of the RGS-containing proteins serve to link other proteins and signaling pathways (Fig. 1). When RGS binds to Ga, it carries with it other functional units providing a great diversity of protein-protein interactions as described in the reviews cited above.

Recent studies suggest evidence for RGS interactions with receptors, providing additional specificity of RGS actions. A PDZ domain in the amino terminus of RGS12 binds selectively with the carboxyl terminus of the IL-8 receptor (Snow et al., 1998). Also, RGS4, in the context of intact cells, can selectively inhibit calcium signals induced by muscarinic versus cholecystokinin receptors, and this specificity is dependent on the amino-terminal extension of RGS4 (Zeng et al., 1998a). Moreover, the specificity of RGS4 for α-subunits is modified in the context of a receptor-Ga fusion protein (Cavalli et al., 2000). All of these results indicate that RGS specificity will depend on factors outside of the RGS-Ga interface.

Furthermore, RGS proteins can accelerate the activation of receptor-stimulated G protein-coupled inwardly rectifying potassium (GIRK) currents (Doupnik et al., 1997; Saitoh et al., 1999) (for more references, see Ross and Wilkie, 2000). The GAP function of RGS explains the temporally enhanced deactivation and activation, but the fact that this acceleration is not accompanied by an expected decrease in the amplitude of stimulated currents suggests that RGS may have additional positive functions in receptor signaling. We recently found (H. Zhong, S. M. Wade, S. M. Wade, 2000).

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2 The abbreviation GAP (for GTPase accelerating protein) is used in several ways that may not please grammarians but does facilitate discussion of RGS function. The noun form “GAP” is commonly recognized and understood. A corruption that greatly simplifies speaking or writing about RGS proteins is the verb form “to GAP”, which means to accelerate GTP hydrolysis. Also, it is occasionally used as an adjective as in “GAP activities” or “non-GAP activities” meaning, respectively, functions that do or do not depend on acceleration of GTP hydrolysis.

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**Fig. 1.** RGS proteins have multiple, independent protein interaction domains that confer unique specificity and functions. In addition to the RGS domain that mediates the interactions with Ga subunits, RGS proteins contain a variety of other protein interaction domains that localize the RGS to specific macromolecular complexes or mediate additional functions. The proteins represented as unfilled shapes illustrate the RGS domain and associated protein interaction motifs for several families of RGS proteins. The characteristics of these RGS families and the individual RGS proteins are outlined in Table 1 and in the text. The proteins shown in the graph are not necessarily oriented from N to C terminus (see Hepler, 1999). (Reprinted from Trends Pharmacol Sci, Vol 20, Hepler, J. R., Emerging roles for RGS proteins in cell signaling, pp 376–382, Copyright (1999), with permission from Elsevier Science.)
and R. R. Neubig, submitted) that RGS4 can enhance α2-adrenergic receptor-stimulated GTPγS binding, acting as a positive kinetic modulator in receptor-G protein coupling. Another possibility is that the GGL domain containing RGS proteins may actually act as Gγ proteins to allow receptor-G protein coupling. The above information implies a direct or indirect interaction of the RGS with receptor as well as with the G protein and provides further support for receptor-specific actions of RGS proteins. These results show that RGS proteins can also enhance receptor signaling, and the net effect must be determined from studies of intact physiological systems.

**Physiological Roles of RGS Proteins**

A large number of studies (reviewed in De Vries et al., 2000) have demonstrated that RGS proteins, when ectopically expressed in mammalian cells, can suppress G protein signaling. In Table 1, we summarize the functional aspects of known RGS proteins. Despite extensive overexpression data, much less is known about the physiological role of endogenous RGS proteins. The model organisms, Saccharomyces cerevisiae and Caenorhabditis elegans, have provided the best evidence for functional roles of RGS proteins. In both cases, loss of RGS protein function leads to hyperstimulation of the signaling pathway, consistent with a primary action via the GAP activity of the RGS protein to suppress G protein signaling.

In mammalian systems, there is little direct information on the roles of endogenous RGS proteins (Table 1). Recently, Jeong and Ikeda (2000) used RGS-insensitive mutants of Gαo (Lan et al., 1989) to show that α2-adrenergic inhibition of N-type calcium currents in rat sympathetic ganglia is markedly inhibited by endogenous RGS proteins. When RGS-insensitive Gαo subunits were expressed, the rate of Ca2+ channel recovery from norepinephrine-induced inhibition was much slower (50–60 s versus 10 s) presumably due to the inability of endogenous RGS proteins to GAP the mutant Gαo. As a critical proof-of-principle for the use of RGS inhibitor drugs, dose-response curves for norepinephrine were left-shifted 6- to 8-fold by expression of the RGS-insensitive Gαo subunits. The effect of these RGS-insensitive Gαo subunit mutations should be the genetic equivalent of blocking the RGS-Gα interaction pharmacologically. Thus RGS inhibitors should lead to enhanced responses to physiologically released or pharmacologically administered agonists. Thus the endogenous levels of RGS proteins, at least in neurons, appear sufficient to influence steady-state ion channel responses through G protein-coupled receptors. The only reported RGS “knockout” shows that endogenous RGS9-1 (alternative splicing product of RGS9 gene, the other product being RGS9-2; see also Table 1) rapidly deactivates transducin in vivo (Chen et al., 2000). In homozygous RGS9-1 knockout mice, the half-time for single photon responses was greatly prolonged (~3 s versus ~0.5 s). An RGS2 knockout exhibits an “anxious” behavioral phenotype, and an RGS14 knockout shows embryonic lethality at the preimplantation stage demonstrating clear functions for these proteins (D. Siderovski, personal communication). Further genetic studies are likely to provide important insights into the physiological functions of different RGS proteins in the near future.

Some additional physiological functions have not yet been proven but could be predicted based on the known biology of RGS protein regulation. The mRNA and/or protein levels of many RGS proteins exhibit rapid induction following physiological signals (for review, see Hepler, 1999; Siderovski et al., 1999; De Vries et al., 2000; Ross and Wilkie, 2000). The yeast RGS protein, Sst2, is up-regulated upon stimulation of yeast by the alpha factor mating pheromone, and this up-regulation leads to a rapid inhibition of pheromone signaling (Dohmlan et al., 1996). Deletion of the SST2 gene leads to a 100-fold enhancement in sensitivity to alpha factor. The mammalian RGS proteins, RGS1 and RGS2, were originally discovered due to the up-regulation of their mRNA levels in immune cells (De Vries et al., 2000). Several studies have demonstrated enhanced mRNA expression of RGS2 by increased cAMP levels (Pepperl et al., 1998) or angiotensin II receptors (Grant et al., 2000; Table 1). Since RGS2 is relatively selective for Gαq, it will be very interesting to determine the role of RGS2 up-regulation in the commonly observed rapid tachyphylaxis to angiotensin II and other activators of phospholipase C (PLC). Additionally, the nuclear localization of some RGS proteins when overexpressed in cells (RGS2, RGS3T, and RGS10) (Chatterjee and Fisher, 2000; Dulin et al., 2000) may suggest a role in regulating gene activation.

RGS proteins may also participate in desensitization or tolerance to opioids (Potenza et al., 1999). Rapid tolerance develops to opioids and many RGS proteins act on the Gαq family G proteins (the main targets of opioid receptors). Thus it will again be very interesting to determine whether RGS proteins play a significant role in opioid desensitization, tolerance, and dependence.

**Physiological Relevance of “Positive” Signaling Properties of RGS Proteins.** Several RGS proteins (such as p115-RhoGEF and Axin) play active roles in transmitting receptor signals to downstream effectors. Several GPCRs can activate Rho including receptors for thrombin (PAR2), lysophosphatidic acid (edg2 and 4), sphingosine-1-phosphate (edg3 and 5), thromboxane A2, and endothelin (Sah et al., 2000). Interestingly, several guanine nucleotide exchange factors for Rho (p115-RhoGEF, PDZ-RhoGEF, and KIAA380) contain an RGS-like domain that selectively interacts with Gα12 or Gα13 (Kozasa et al., 1998). In particular, purified p115-RhoGEF binds to and stimulates the GTPase activity of both Gα12 and Gα13, and GTPγS-bound Gα13 stimulates activity of purified p115-RhoGEF (Hart et al., 1998; Kozasa et al., 1998). Thus, p115-RhoGEF appears to serve as a direct effector for Gα13, transferring the signal from a GPCR-activated heterotrimeric G protein to the low-molecular weight G protein, Rho. This mechanism fits very well with the large body of literature showing that activation of Gα12 and Gα13 causes Rho-dependent changes in cell shape and growth properties (Sah et al., 2000).

Axin, a component of the Wnt signaling system important in embryonic development, organogenesis, and cancer (Wodarz and Nusse, 1998; Peifer and Polakis, 2000), contains a functionally important RGS domain. Signals from the ligand (Wnt) and receptor (Frizzled) to downstream components such as Axin, glycogen synthase kinase 3 (GSK3), adenomatous polyposis coli protein (APC), and β-catenin are poorly understood. APC is a known tumor suppressor, and β-catenin is an oncogene. Axin, in complex with APC and GSK3, negatively regulates the transcription factor β-cate-
TABLE 1
Examples of the functional effects exerted by members of the various RGS subfamilies in different systems
Mammalian and nonmammalian RGS proteins are both listed and are grouped by characteristic structural domains. See other reviews mentioned in the text for references on the molecular sizes, domains, and tissue distribution of individual RGS proteins.

<table>
<thead>
<tr>
<th>RGS</th>
<th>Size (aa)</th>
<th>Non-RGS Domains</th>
<th>Tissue Distribution</th>
<th>Examples of Functional Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedicated/small RGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RGS1</td>
<td>196h</td>
<td>Short N-terminal domain, function not determined</td>
<td>Lymphocytes</td>
<td>Down-regulates chemotaxis of lymphocytes to chemokines (Moratz et al., 2000); modulates postsynaptic GIRK currents in neurons and atrial cells (Doupnik et al., 1997);</td>
</tr>
<tr>
<td>RGS2</td>
<td>211h</td>
<td>Ubiquitous</td>
<td>Ubiquitous</td>
<td>Suppresses mGluR1a-mediated inhibition of M-type potassium currents in sympathetic neurons (Kammermeier and Ikeda, 1999); inhibits muscarinic acetylcholine receptor-mediated MAP kinase activation (Ingi et al., 1998). Knockout mice show reduced T cell proliferation and IL-2 production, increased anxiety responses, and decreased male aggression (Oliveira-Dos-Santos et al., 2000). Functions are also implied by its dynamic regulation in osteoblasts as well as by its increase in expression in response to stimuli such as elevated cAMP levels, PKC, neuronal activity, and certain psychoactive agents (Ingi et al., 1998; Burchett et al., 1999).</td>
</tr>
<tr>
<td>RGS8</td>
<td>180r</td>
<td>Brain</td>
<td>Brain</td>
<td>Accelerates GIRK currents (Saitoh et al., 1999); attenuates receptor-mediated Ca\textsuperscript{2+} channel inhibition in HEK293 cells.</td>
</tr>
<tr>
<td>RGS10</td>
<td>173h</td>
<td>Brain</td>
<td>Brain</td>
<td>Attenuates angiotensin receptor-mediated IP production and M1 muscarinic receptor-stimulated gene activation via cAMP-responsive element (Park et al., 2000).</td>
</tr>
<tr>
<td>RGS13</td>
<td>159h</td>
<td>Lung</td>
<td>Lung</td>
<td>Attenuates platelet-activating factor-induced responses in CHO cells; inhibits translocation of GLUT4 in adipocytes (Kanzaki et al., 2000); potential function is suggested by increased expression of RGS16 in response to genotoxic stress (Buckhinder et al., 1997) and by its possible mutation contributive to certain forms of retinitis pigmentosa (Bessant et al., 2000).</td>
</tr>
<tr>
<td>RGS18</td>
<td>235m</td>
<td>Bone marrow, lung, spleen, fetal liver</td>
<td>Brain, heart</td>
<td>Down-regulates chemotaxis of lymphocytes to chemokines; modulates GIRK currents in neurons and atrial cells (Doupnik et al., 1997); inhibits translocation of the glucose transporter (Glut4) in adipocytes (Kanzaki et al., 2000).</td>
</tr>
<tr>
<td>RGS4</td>
<td>205r</td>
<td>Short N-terminal domain with amphipathic helices important for membrane association</td>
<td>Brain, heart</td>
<td>Attenuates angiotensin receptor-mediated IP production and M1 muscarinic receptor-stimulated gene activation via cAMP-responsive element (Park et al., 2000).</td>
</tr>
<tr>
<td>RGS5</td>
<td>181m</td>
<td>Brain, heart, lung, muscle</td>
<td>Liver, pituitary</td>
<td>Attenuates angiotensin receptor-mediated IP production and M1 muscarinic receptor-stimulated gene activation via cAMP-responsive element (Park et al., 2000).</td>
</tr>
<tr>
<td>RGS16</td>
<td>201m</td>
<td>Long N-terminal domain, function not determined</td>
<td>Kidney</td>
<td>Down-regulates chemotaxis of lymphocytes to chemokines; modulates postsynaptic GIRK currents in neurons and atrial cells (Doupnik et al., 1997); reduces tubular migration in kidney (Gruning et al., 1999); attenuates receptor-mediated Ca\textsuperscript{2+} channel inhibition in HEK293 cells.</td>
</tr>
<tr>
<td>Dedicated RGS?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RGS3</td>
<td>519h</td>
<td>Long N-terminal domain, function not determined</td>
<td>Kidney</td>
<td>Down-regulates chemotaxis of lymphocytes to chemokines; modulates postsynaptic GIRK currents in neurons and atrial cells (Doupnik et al., 1997); reduces tubular migration in kidney (Gruning et al., 1999); attenuates receptor-mediated Ca\textsuperscript{2+} channel inhibition in HEK293 cells.</td>
</tr>
</tbody>
</table>

Cysteine string RGS |
| GAIP     | 217h      | N-terminal cysteine string | Heart, liver, lung | Possible role in the regulation of vesicular trafficking (De Vries et al., 1998). |
| Ret-RGS1 | 374b      | Retina              | Retina              | Regulates egg-laying behavior (Koelle and Horvitz, 1996). |
| RGS-Z1   | 217h      | Brain               | Brain               | Inhibits endogenous Gq/G11 activity in COS-7 cells (Hajdu-Cronin et al., 1999). |
| RGS17\textsuperscript{c} | 210c | Not determined | | |

GoLoco RGS |
| RGS12   | 1387r     | Raf-I, GoLoco, PDZ/PTB | Brain, lung, liver | Inhibits Go12/Go13-mediated serum response factor activation in NIH3T3 cells; attenuates IL-8 receptor-mediated MAP kinase activation and M1 acetylcholine receptor-stimulated e-fos SRE activation (Cho et al., 2000). |
| RGS14   | 547m      | Raf-I, GoLoco       | Brain, lung, spleen | |
| RGS17\textsuperscript{d} | 217h | Brain               | Brain, liver, lung | Generally viewed as a tumor suppressor (Peifer and Polakis, 2000). |

Axin RGS |
| Axin    | 832r      | GSK-β3/β-catenin binding; DIX | Ubiquitous | |
| Conductin | 840m     | Brain, liver, lung | | |
nin, in part by causing its ubiquitination and degradation (Wodarz and Nusse, 1998). Wnt binding to its receptor, Frazzled, relieves this inhibitory effect thus increasing levels of active β-catenin. The recent crystal structure of an Axin/APC complex revealed that APC binds to the RGS domain of Axin (Spink et al., 2000). Interestingly, the site of the Axin-APC interaction on the RGS domain is distinct from the site at which Ga subunits bind to RGS proteins (Fig. 2). Based on these structures, it would be appropriate to define at least two interaction sites on the RGS domain—an “A-site” for Ga subunit and a “B-site” on the back of the RGS domain where APC binds Axin. This provides two distinct pharmacological targets on the RGS domain that may exhibit different pharmacological responses. The A-site on Axin, however, is only hypothetical since the RGS domain of Axin has not yet been reported to act on a Ga as other RGS proteins do.

Another recent observation related to the B-site has to do with PIP₃ as a potential physiological inhibitor of RGS (Popov et al., 2000). PIP₃ binds to RGS4 and RGS16 and inhibits RGS4 GAP activity. This appears to involve α helix 5 of RGS4 in the B-site. Furthermore, calmodulin binds to the same site and relieves the PIP₃-mediated inhibition. This potential physiological regulatory mechanism could be a useful target for therapeutic intervention as discussed below.

It is clear that RGS proteins do play important physiological roles in G protein function, some fairly predictable based on existing information. Other functions are likely to involve novel interactions and/or mechanisms. Thus, we are just scratching the surface in defining the physiological roles of RGS proteins, and many completely unpredictable actions will probably be revealed over the next few years.

Possible Therapeutic Uses of RGS-Targeted Drugs

In thinking about therapeutic uses of RGS-directed drugs, we must consider site of action on the protein, cellular mechanism, and relation to (or interaction with) known pharmacological agents.

Sites of Action

RGS A-Site. The most obvious drug design would be an inhibitor of RGS interaction with Ga subunits. We denote this site on RGS the A-site. Such a drug would be expected to block RGS actions that depend on Ga binding. Inhibition of the GAP activity should increase the effect of the Ga subunit and increase the sensitivity of any tissue expressing that RGS protein to agonists activating that G protein. This is similar to agonist potentiators such as the benzodiazepines at the GABA-A receptor. In addition to the classical effect of RGS as a GAP, some RGS domains are also effector antagonists or can presumably localize other inhibitory molecules (e.g., GRK2 or GRK3) to the site of receptor-activated G proteins. In both of these cases, a drug binding to the RGS A-site would potentiate agonist signaling by both endogenous and exogenous agonists.

For an RGS that serves as an effector (e.g., p115-RhoGEF), an RGS A-site inhibitor would be an antagonist of that particular pathway. Since Rho activation is involved in cell proliferation (Peifer and Polakis, 2000) and metastasis (Clark et al., 2000), this could represent a useful site for anticancer or antimetastatic agents (see below). Thus, RGS A-site inhibi-
tors could either lead to enhancement or inhibition of the signaling pathway, depending on the specific role of that RGS protein.

RGS B-Site. Drugs acting at the RGS B-site could potentially serve either as RGS inhibitors or as RGS activators. In the Wnt signaling system, a drug that could inhibit β-catenin function could be very useful in cancer therapy. An Axin B-site inhibitor would block the Axin-APC interaction and inhibit the ability of the Axin/APC/GSK3 complex to downregulate β-catenin. Unfortunately, β-catenin is oncogenic, so this would not be a desirable action for developing anticancer agents. If a G protein α-subunit binding to the A-site on the Axin RGS domain stimulates APC release from the B-site, then inhibition at the Axin RGS A-site might be useful for cancer therapy by disrupting growth signals from the Wnt pathway.

A potentially novel effect of a drug at the RGS B-site would be to serve as an “RGS agonist”. If endogenous lipid mediators suppress RGS action, then preventing the binding of that lipid would stimulate RGS function and decrease signaling through the pathway. Such a drug would act in a manner similar to that proposed for the endogenous regulator calmodulin (Popov et al., 2000). Such an RGS agonist could lead to reductions in Goa, Gai, or Gag signaling.

Other Domains. One aspect of RGS proteins that differentiates them from G proteins as potential drug targets is their incredible structural diversity. As noted in Table 1, there are a number of other interaction motifs beyond the RGS domain that could be targeted in drug design. This could include the DH/PH domains of the p115-RhoGEF family, the AKAP domain of D-AKAP2, and the PDZ domain of RGS12, which targets IL-8 receptors (Snow et al., 1998a). Specifically, inhibitors of RGS12 PDZ domain could produce more specific effects than inhibitors of the RGS domain itself. This would result from the lack of effect of a PDZ inhibitor on the short forms of RGS12 (which lack the PDZ domain), and such an inhibitor would also only affect those receptors that were directly targeted by RGS12 localization. Further discussion, however, will focus on drugs targeting the RGS domain itself, either on the A-site or the B-site.

Potential Clinical Uses of RGS Inhibitors

There are at least four different ways in which RGS inhibitors could be used either alone or in combination with other drugs: 1) RGS inhibitors could be used as potentiatius of endogenous agonist function similar to the action of benzodiazepines at the ionotropic GABA-A receptor (Macdonald and Olsen, 1994). 2) They could also be used to potentiate the action of or block desensitization to exogenously administered GPCR agonists. This may be especially useful in the case of agonists for which rapid desensitization of responses occurs such as opioid analgesics. 3) They could be used to modify and/or increase the specificity of an exogenously administered agonist. 4) They could block effector signaling by an RGS protein (e.g., Rho GEF or APC activation). The inhibitor could be targeted to the RGS domain itself or to other domains that are critical for interactions with other signaling molecules (e.g., PDZ or GEF domains).
**Endogenous Agonist Potentiators.** The dramatic increase in sensitivity to epinephrine-mediated Ca\(^{2+}\) channel inhibition in rat superior sympathetic ganglion neurons (Jeong and Ikeda, 2000) when RGS function is abrogated suggests that RGS antagonists could significantly enhance the function of endogenous neurotransmitters. This would mainly occur for receptors coupled to G\(\alpha_i\) or G\(\alpha_q\) signaling pathways as there is no strong evidence for an RGS effect on a G\(\alpha_o\) family member. Thus, actions of the inhibitory adrenergic receptors (\(\alpha_2\)-adrenergic receptors) on cAMP levels would be enhanced at the expense of the stimulatory adrenergic receptors (\(\beta\)-adrenergic receptors). A particularly striking example in which an RGS inhibitor might be more specific than a receptor agonist is RGS9-2, which is highly localized in the caudate putamen (Gold et al., 1997). An RGS9-2 inhibitor could enhance D2 dopamine action with potential anti-Parkinsonian effects. Similarly, RGS inhibitors targeting brain regions involved in pain control such as the peri-aqueductal gray region (e.g., RGS8, RGS7, or RGS4) might serve as novel analgesics or analgesic potentiators. If regions involved in reinforcement behaviors such as ventral tegmental area or nucleus accumbens had less of this RGS protein (e.g., RGS4 or RGS7), then the actions of opioids could target the desired analgesic effect while reducing potential dependence liability. Similarly, many receptors acting through G\(\alpha_o\)- or G\(\alpha_i\)-mediated signaling pathways (e.g., GABA-B receptors for muscle relaxants) could be potentiated in a tissue-specific manner by an RGS inhibitor.

PLC is another potential target (e.g., serotonin agonists for treatment of migraine (Diener et al., 1999)). \(G_{\alpha}\) responses in cerebral vascular tissue (Grant et al., 2000) could be selectively potentiated depending on RGS proteins present in that tissue. Another intriguing but speculative possibility is suggested by the evidence that expressed RGS4 and RGS16 block translocation of the glucose transporter (Glut4) to the plasma membrane in 3T3-L1 adipocytes (Kanzaki et al., 2000; Table 1). If an endogenous RGS tonically inhibits Glut4 translocation in vivo, then an inhibitor of that RGS could potentially be used in the therapy of type II diabetes. RGS1 inhibits signaling by platelet activating factor, stromal-derived factor-1, and constitutively activated Ga\(\alpha_{12}\) (Moratz et al., 2000; Table 1). An inhibitor of RGS1 might be a novel immune stimulant for use in acquired immunodeficiency syndrome or cancer by activating germinal center B lymphocytes, which are normally refractory to stromal-derived factor-1-triggered migration.

**Combination Exogenous Agonist/RGS Inhibitor Therapy.** The combination of a classical GPCR agonist with an RGS inhibitor could: 1) potentiate the drug’s effect as described above, 2) reduce desensitization and/or drug tolerance, or 3) target responses to particular tissues to reduce side effects. The practicality of the second effect will depend on the contribution of RGS proteins (as opposed to receptor kinases) in the desensitization process. Both mechanisms 1) and 2) are illustrated in the phenotype of yeast strains lacking Sst2p since they are sensitive to much lower doses of pheromone than are wild-type strains, plus they display a persistent growth-arrest in response to pheromone, while the wild-type strains rapidly recover from growth-arrest.

An RGS inhibitor could provide a tissue-specific targeting of the action of an agonist that stimulates many different tissues. A major problem with apomorphine or other D2 dopaminergic agonists in Parkinson’s disease is side effects on peripheral tissues. Apomorphine is given with a peripherally acting D2 blocker to reduce these side effects (O’Sullivan and Lees, 1999). Alternatively, an inhibitor of RGS9-2 could be given with apomorphine or other D2 agonists to potentiate agonist effects in the desired tissue (caudate putamen) thus improving selectivity by reducing undesirable effects occurring in other tissues.

In addition to tissue-specific effects, RGS inhibitors could provide pathway-directed targeting of a drug responses. A muscarinic agonist for treatment of Alzheimer’s disease (which has side effects on many tissues) could be combined with an inhibitor of an RGS in the brain region of interest to locally potentiate the agonist action. Depending on the nature of the RGS proteins present, it may also be possible to direct the agonist action to particular signaling pathways. Specifically, RGS2 has greater inhibitory effects on G\(\alpha_q\) and PLC responses than on G\(\alpha_o\) or G\(\alpha_{12}\) responses (Heximer et al., 1999). The M1, M3, and M5 muscarinic receptors activate G\(\alpha_q\), while M2 and M4 activate G\(\alpha_o\) and G\(\alpha_i\). Thus an RGS2 inhibitor would be expected to selectively enhance responses to the PLC-coupled M1, M3, and M5 receptors and could increase the selectivity of a partially selective M1 muscarinic agonist for M1 versus M2 receptor responses.

**Blocking RGS-Mediated Effector Function.** The role of a RhoGEF RGS domain in receptor-stimulated Ga\(\alpha_{12}/Ga\alpha_{13}\)-mediated Rho activation is of potential significance. Ga\(\alpha_{12}\) and Ga\(\alpha_{13}\) can be oncogenes (Gutkind, 1998). Also, edg receptors stimulate cellular proliferation in response to serum-derived lipid signals such as lysophosphatidic acid and sphingosine-1-phosphate and are present in many cancer cell types such as breast, colon, lung, and melanoma (Fang et al., 2000). The leukemia-associated RhoGEF gene LARG has an RGS homology region as in p115-RhoGEF (Kourlas et al., 2000). Finally, a central role for RhoC in metastasis has just been identified (Clark et al., 2000). Thus drugs decreasing RhoGEF activity could be very useful anticancer agents. The RhoGEF domain is a compelling drug target, but this may have nonspecific effects. An inhibitor directed at the RGS domain of the p115-RhoGEF (or its homologs PDZ-RhoGEF, lsc, LARG, etc.) could block the effects of lipid mediators or other G protein-mediated signals to cell growth, anti-apoptosis, or metastasis. Targeting the RGS region of RhoGEFs may prove more specific and less toxic, at least in cancers where the growth stimulus involves a G protein signaling pathway, since this does not affect other RhoGEF subtypes in the genome that don’t contain an RGS domain.

**Potential Clinical Uses of “RGS Agonists”**

The concept of RGS agonists is more speculative but is worth considering. Blocking interactions of endogenous inhibitors of RGS function such as PIP\(_3\) (see above) could serve to stimulate the GAP activity of RGS proteins leading to reductions in G\(\alpha_o\), G\(\alpha_i\), or G\(\alpha_q\) signaling. Since many stimulatory ligands (e.g., chemokines, epinephrine, angiotensin, and endothelin) act through G\(\alpha_i\)- or G\(\alpha_q\)-stimulated phospholipase C activity, a drug that could suppress signaling in selective tissues could be very useful. Examples could include: 1) inflammatory conditions in which an RGS1 stimulator would block G\(\alpha_i\) responses, and 2) hypertension and vascular restenosis in which an RGS2 stimulator could block G\(\alpha_q\) signals. Development of this area will require additional
understanding of the tissue localization, specificity, and basic regulatory processes governing the function of RGS proteins.

Conclusions and Future Directions

Clearly, much more will be learned about RGS physiological functions, specificity, cellular and tissue localization, and potential as drug targets. Currently, this is a very exciting aspect of G protein-co coupled receptor signaling. Given the critical importance of G proteins in physiological processes and their receptors as a locus of action for many useful therapeutic agents, it is highly likely that RGS proteins will provide new opportunities for drug development and specificity. Stay tuned!

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