Neuron, Vol. 38, 853–856, June 19, 2003, Copyright ©2003 by Cell Press

From Molecules to Behavior: New Clues for RGS Function in the Striatum

Minireview

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RGS proteins act as negative regulators of G protein signaling, and there is growing evidence that the RGS family is important for regulating signaling in neurons. Two articles in this issue of *Neuron* (Martemyanov et al. and Rahman et al.) shed light on the function of one family member, RGS9-2, in behavioral responses to dopamine signaling in the striatum and on the relationship between its structure and its function.

In recent years, RGS (Regulators of G protein Signaling) proteins have emerged as key components in regulation of cellular responses to extracellular signals acting through G protein-coupled receptors (reviewed in Hollinger and Hepler, 2002). The RGS domains of this diverse family, encoded by close to 30 genes in mammals, bind to α subunits of heterotrimeric G proteins and accelerate their hydrolysis of GTP, thus speeding their return to the inactive state and terminating or attenuating downstream responses. They serve as GTPase Accelerating Proteins, or GAPs. Although many neuromodulators are generally considered to be slow acting, the abundance of some members of the RGS family in the nervous system suggests that neurons commonly require temporal regulation of intracellular G protein signaling. Although RGS proteins are differentially expressed in region-specific patterns in the mammalian brain, defining the precise physiological roles of individual RGS proteins in neurons has, with a few notable exceptions, remained an elusive problem.

One exception has been RGS9-1, one of two products of the RGS9 gene. Its role as the regulator of G protein inactivation kinetics during the recovery phase of light responses in rods and cones has been firmly established by a combination of biochemical and physiological experiments with wild-type and knockout animals. Understanding the physiological function of RGS9-1 in photoreceptors has facilitated studies of its regulation and led to the discovery of its interaction with other signal transducing proteins besides its target $G_{\alpha t}$, including its obligate binding partner $G_{\beta 5}$, an anchor protein R9AP, a cGMP phosphodiesterase inhibitory subunit, $PDE6_{\gamma}$, and protein kinase C.

RGS9-1 is a member of the R7 (or C) family of RGS proteins, which share a common multidomain structure (Zhong and Neubig, 2001; Cowan et al., 2001) including, near the N terminus, a DEP domain, an intervening or I domain, a G protein γ-like (GGL) domain, and the catalytic core RGS domain (Figure 1). The functions of the DEP and I are not completely understood, although there is evidence supporting a role in membrane targeting, and the GGL domain mediates specific interaction with a G protein β subunit, $G_{\beta 5}$.

There are four genes encoding mammalian R7 family members, *RGS6*, *RGS7*, *RGS9*, and *RGS11*, which through alternative RNA processing and posttranslational modifications give rise to over a dozen different proteins. For example, a brain-specific isoform of RGS9, RGS9-2, has aroused particular interest because its expression is largely restricted to the dorsal and ventral striatum, which receive most of the inputs to the basal ganglia. RGS9-2 is expressed in the medium spiny cells, upon which many neuromodulatory afferents converge to control the output to the globus pallidus and substantia nigra (reviewed in Greengard, 2001; Calabresi et al., 2000). Despite the fact that the anatomical and physiological properties of these neurons within the circuitry of the basal ganglia are fairly well understood, the importance of terminating G protein signaling rapidly—and thus the specific role of RGS9-2 in these cells—remains unknown. Two papers in this issue of *Neuron* (Martemyanov et al., 2003 and Rahman et al., 2003) provide new clues to the function and regulation of RGS9-2. Readers interested in the previously published work on RGS9-1 and RGS9-2 are referred to the citations in these two papers, including RGS9-specific reviews (e.g., Arshavsky et al., 2002; Cowan et al., 2001).

Much about the structure and function of RGS9-2 has been learned by comparison to the biochemical properties of RGS9-1 (Figure 1B). Like RGS9-1, RGS9-2 also contains a GGL domain, which mediates its interaction with $G_{\beta 5S}$, a splice variant of the $G_{\beta 5}$ gene expressed in brain. The association of RGS9-2 with $G_{\beta 5}$ is important for the stability and GAP activity of the complex, similar to the effects of $G_{\beta 5L}$ on RGS9-1. One of the more intriguing features of RGS9-1 is its regulation by the γ subunit of the effector enzyme cGMP phosphodiesterase (PDE6), which is activated by RGS9-1's target G protein transducin ($G_{\alpha t}$). The $G_{\alpha t}$ complex with PDE_{γ} is a much better (lower K_m) substrate for RGS9-1 than is the G protein itself. The mechanism for this effect is complex; mutagenesis studies with full-length and truncated forms of RGS9-1 reveal that its multiple domains, as well as its obligate binding partner $G_{\beta 5}$, all play important roles in the regulation by PDE_{γ}. The domains important for stimulation of RGS9-1's GAP activity by PDE_{γ} include the short, distal C-terminal domain of RGS9-1 that is absent in the brain isoform RGS9-2. RGS9-2 has instead a completely different and much longer C-terminal domain (Figure 1), for which until recently there were no functions known.

These observations have raised the question of how RGS9-2 can serve as an effective GAP for its target G protein(s) in the absence of PDE_{γ}. Traces of PDE_{γ} expression have been found in other tissues such as lung, but none so far in the striatum where RGS9-2 expression is largely confined. A partial answer to this

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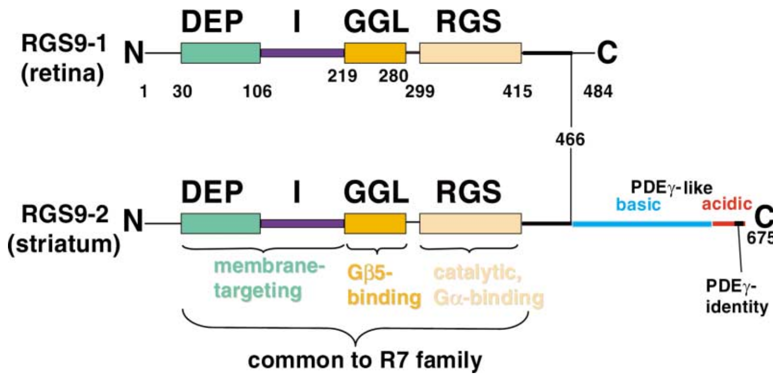

Figure 1. Domain Structure of RGS9-1 and RGS9-2

question is provided by recent results from Vadim Arshavsky and colleagues (Martemyanov et al., 2003). They noticed sequence similarity between part of the C-terminal tail of RGS9-2 and PDE_{γ} and decided to test the hypothesis that this domain increases RGS9-2 GAP activity, analogous to the effect of PDE_{γ} on RGS9-1. The results suggest that indeed it does. In the presence of the PDE_{γ}-like stretch in its C-terminal tail, RGS9-2 displays high GAP activity, just as RGS9-1 does in the presence of PDE_{γ}. Addition of PDE_{γ} does not stimulate GAP activity further, but instead inhibits it, presumably by competing with RGS9-2's C-terminal tail. When GAP activity was tested for a truncated form of RGS9-2 lacking the C-terminal 45 amino acids, including a stretch of six identical to a sequence in PDE_{γ}, the activity was significantly reduced. Taken together, these observations seem to be yet another example of a principle pointed out in genome-scale studies from David Eisenberg and colleagues: pairs of proteins homologous to pairs of domains found covalently attached in multidomain proteins are very often functional interaction partners (Marcotte et al., 1999). As pointed out by Martemyanov et al., one function of such interaction partners can be to act as "affinity adapters" to control specificity of interactions with additional proteins. However, the details of the interactions seem to be somewhat different in the two cases compared here. Six consecutive residues in PDE_{γ} are known by crystallography to contact G_{α} in the G_{α}-RGS-PDE_{γ} complex (Slep et al., 2001), and mutating one of them, Trp70, causes complete loss of the ability to stimulate GAP activity (Slepak et al., 1995). Changing all six to alanines in RGS9-2 is apparently without effect. In the course of these studies, Martemyanov et al. also found that RGS9-2 is a better GAP for $G_{\alpha o}$ than $G_{\alpha i1}$, as reported previously for RGS9-1 (Hooks et al., 2003), and found that RGS9-2, like RGS9-1, is an even better GAP for $G_{\alpha t}$. Given that $G_{\alpha t}$ and RGS9-2 are not found in the same cell types, it seems likely that localization plays a more important role in determining specificity of action of RGS isoforms on different G proteins than the differences in intrinsic affinity observed.

Important clues to the function of RGS9-2 have come from behavioral experiments by Eric Nestler and colleagues (Rahman et al., 2003 [this issue of *Neuron*]) that utilize motor activity and reward-seeking as measures of dopaminergic signaling in the ventral striatum. Viral-mediated overexpression of either RGS9-1 or RGS9-2 isoforms unilaterally in the nucleus accumbens induced a profound circling toward the side with RGS9-2 overexpression when the rats were administered dopamine receptor (specifically D2) agonists. This circling behavior is thought to arise from an imbalance in dopaminergic signaling, consistent with the overexpression of RGS9-2 decreasing the sensitivity to dopamine. Rats overexpressing RGS9-2 bilaterally showed less sensitivity to moderate doses of cocaine, as assayed by a reduction in cocaine-induced locomotor activity. Conversely, RGS9 knockout mice show enhanced locomotor activity, greater sensitization, and greater place conditioning in response to cocaine. Together, these results are consistent with the notion that RGS9-2 turns down the response to dopamine in the nucleus accumbens.

The results from Nestler's group imply a role for RGS9-2 in signaling from D2 dopamine receptors, but it is too early to say whether the effects they observe are the result of direct action on G proteins activated directly by D2 receptors or the result of a more complex network of interactions (Figure 2). Because RGS9-2 does not need to rely on an effector molecule to enhance GTP hydrolysis (see above), it remains unknown how and if the action of RGS9-2 is cascade specific. Answering these questions will require knowing which G proteins and effectors, operating in response to which G protein-coupled receptors, are regulated by RGS9-2. Localization of RGS9-1 in rod outer segments where

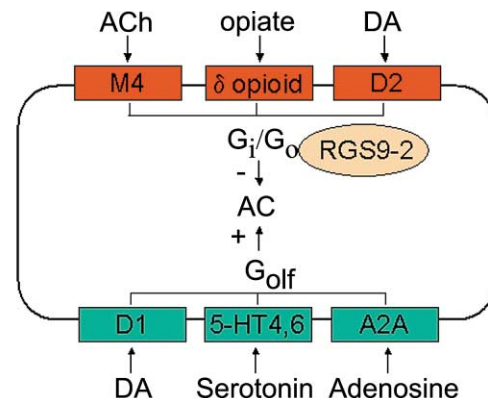

Figure 2. Convergent Regulation of Adenylate Cyclase Activity by Neuromodulators in Medium Spiny Cells of the Striatum

D1 and M4 receptors are usually expressed in striatonigral (Substance P-containing) neurons, whereas D2 and A2A are mostly expressed in striatopallidal (enkephalin-containing) neurons.

rhodopsin and transducin are found played an important role in determining its function. Unfortunately, precise colocalization in striatal neurons has been difficult because the several antibodies used to great effect with RGS9-1 suffer from low specificity and affinity when applied to RGS9-2 in striatal extracts. Localization is critical because RGS9-2 presumably must be colocalized with the G proteins and effectors it regulates, which in turn are likely colocalized with one or more specific GPCR. Moreover, it has been reported that RGS9-2 and other R7 RGS proteins can be found in the nucleus, but the role of the nuclear pool in G protein signaling is unknown. Although RGS9-2 mRNA has been identified in medium spiny neurons expressing dopamine D2 receptors, it is also present in an equal fraction of cells that express D1 receptors (Rahman et al., 2003). Medium spiny cells can be roughly divided into two populations, those that contain substance P and primarily express D1, and those that contain enkephalin and primarily express D2. The expression of RGS9 in both of these cell types suggests that its function may not be to regulate D2 receptor signaling per se, but to regulate the cascade of some other G protein-coupled receptor that also regulates adenylate cyclase activity (Figure 2) and is present in both cell populations.

On the other hand, the actions of RGS proteins may be extraordinarily specific for a particular GPCR-coupled cascade, particularly if they exist in specific, supramolecular complexes. Indeed, some RGS proteins appear to be able to directly regulate GPCRs and effectors (Druey, 2001). For example, non-R7 RGS proteins such as RGS2 can directly inhibit adenylate cyclase (reviewed in Kehrl and Sinnarajah, 2002), and an RGS12 isoform directly interacts with the C terminus of the interleukin-8 receptor B/CXCR2 in vitro (Snow et al., 1998). Similar receptor-RGS interactions have been proposed for the R7 family, including RGS9. Current evidence for such interactions for RGS9-2 is indirect and largely based on the lack of effect of RGS proteins on the steady-state activation of GIRK channels in oocyte expression systems. However, in the present study (Rahman et al., 2003), coexpression of RGS9-2 with $G_{\beta 5S}$ in oocytes did result in a significant reduction in the steady-state current amplitude, consistent with the idea that RGS9-2—which is stabilized by the presence of $G_{\beta 5S}$—acts solely by stimulating GTP hydrolysis. Similar effects on the amplitude of GIRK channels by Go and RGS7 in the presence and absence of $G_{\beta 5}$ have also been reported (Zhang et al., 2002). If indeed D2 activation of G_i/G_o is regulated by RGS9-2 directly, then other G_i/G_o-coupled pathways (such as signaling through M4 acetylcholine receptors) should be unaffected by the loss of RGS9. Crossing the RGS9 knockouts to existing mice lacking various GPCR subtypes will be helpful in determining this specificity.

A remaining question involves the functional importance of RGS9-2's long C-terminal tail. The biochemical studies suggest that activation of RGS9-2 in the striatum may be constitutive because of the ability of the C-terminal extension to supply the activity enhancement supplied by PDE_γ in the retina. However, RGS9-1, which has low activity in the absence of PDE_γ, is nearly as effective in behavioral responses to drug treatment as RGS9-2 when ectopically expressed in the striatum. In light of the biochemical evidence presented by Martemyanov et al., one might expect that RGS9-1 should have lower affinity for its target (presumably G_o) than RGS9-2, at least in the absence of PDE_γ. Perhaps the native substrate for RGS9-2 in these cells is normally a low-affinity interaction, such as that with $G_{\alpha i}$, which Martemeyanov et al. found to be relatively insensitive to both RGS9-1 and RGS9-2. Alternatively, perhaps an additional molecule in the G protein/effector complex can serve this affinity adaptor function in the absence of the C-terminal tail normally present on RGS9-2. A more mundane explanation may be that the levels of RGS9-1 were sufficiently higher than normal cellular levels of RGS9-2 to act effectively despite its lower basal activity. The fact that overexpression of RGS4, which shows robust GTPase activity for both Go and Gi, had no effect suggests that interactions with other proteins, likely through the non-RGS domains, are important for RGS9-2 function.

Another puzzle is the role of $G_{\beta 5}$ in the striatum. Striatal neurons express a short version of this protein, $G_{\beta 5S}$, and although $G_{\beta 5S}$ potentiates the effects of RGS9 in vitro, efficacy of RGS9-2, presumably due to GAP activity, can be observed in the absence of $G_{\beta 5S}$ (Kovoor et al., 2000; Rahman et al., 2003). However, recent results with $G_{\beta 5}$ knockout mice (Chen et al., 2003) support earlier proposals that $G_{\beta 5}$ is essential for stable expression of RGS9 and other R7 family members. It is thus surprising that overexpression of both RGS9 isoforms without simultaneous overexpression of $G_{\beta 5}$ has potent effects on behavioral responses to drugs. The ability to obtain overexpression of RGS9-2 and RGS9-1 in the striatum may be due to endogenous expression of $G_{\beta 5}$ exceeding that of RGS9-2, or to some other GGL binding protein compensating for the relative $G_{\beta 5}$ insufficiency.

It is interesting to note that chronic cocaine administration increased RGS9-2 protein levels (Rahman et al., 2003), yet had no effect on RGS9 mRNA (Taymans et al., 2003; Rahman et al., 2003). This suggests a posttranscriptional mechanism can regulate the levels of RGS protein in response to agonist exposure. Similarly, in photoreceptors, disruption of either RGS9 (Chen et al., 2000) or $G_{\beta 5}$ (Chen et al., 2003) genes causes a loss of $G_{\beta 5L}$ and RGS9-1 proteins, respectively, without corresponding changes in mRNA levels in either case. Thus, the posttranscriptional regulation of RGS9—and perhaps other RGS proteins—seems an important area for future study.

The fact that overexpression or loss of expression of a single protein that controls G protein deactivation has dramatic effects on drug-induced behavior challenges the notion that dopamine and other neuromodulators are slow acting. If the purpose of RGS9-2 was simply to reduce steady-state signaling, changing surface receptor expression seems a simpler approach. Instead, the ability of RGS9-2 to modulate dopamine's effects suggests that the cell needs to diminish its response to dopamine actively. Such rapid and dynamic control of G protein signaling has precedent; in photoreceptors, for example, RGS9-1's action becomes apparent about 100 ms following activation of the receptor protein, rhodopsin (Chen et al., 2000). The apparent importance and behavioral relevance of controlling the duration of G protein signaling suggests that the actual time course

of G protein signaling in the striatum—and elsewhere in the brain—requires closer examination.

Although dopamine signaling can be affected by the levels of expression of RGS proteins (and vice versa), the steps linking molecules to behavior remain undefined. From the present results, it is clear that RGS proteins play an important role in GPCR modulation of neuronal signaling in vivo and that this role is specified by complex interactions with multiple proteins and adaptor domains. It therefore seems likely that other members of the RGS family will also be implicated in temporal regulation of neuronal signaling in other discrete brain regions, and as such are attractive targets for psychotropic drugs. A "vertical" approach that merges structure/function studies like those of Arshavsky with behavioral assays like those of Nestler, and with combined physiological and molecular studies at the single-cell level, is needed to determine the specificity with which RGS proteins regulate G protein signaling in vivo. Integration of the results from this approach should allow us to understand how RGS proteins write the spatial and temporal rules by which neuromodulators like dopamine exert their effects.

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