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Issue: *Addiction Reviews 2***Regulator of G protein–signaling proteins and addictive drugs**

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Regulator of G protein–signaling (RGS) proteins are a family of more than 30 intracellular proteins that negatively modulate intracellular signaling of receptors in the G protein-coupled receptor family. This family includes receptors for opioids, cannabinoids, and dopamine that mediate the acute effects of addictive drugs or behaviors and chronic effects leading to the development of addictive disease. Members of the RGS protein family, by negatively modulating receptor signaling, influence the intracellular processes that lead to addiction. In turn, addictive drugs control the expression levels of several RGS proteins. This review will consider the distribution and mechanisms of action of RGS proteins, particularly the R4 and R7 families that have been implicated in the actions of addictive drugs, how knowledge of these proteins is contributing to an understanding of addictive processes, and whether specific RGS proteins could provide targets for the development of medications to manage and/or treat addiction.

**Keywords:** drugs of abuse; opioids; stimulants; opioid receptors; dopamine receptors; RGS proteins; G proteins; cell signaling

**Introduction**

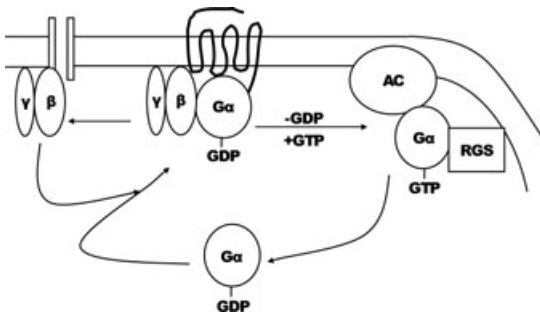
Regulator of G protein–signaling (RGS) proteins are a family of more than 30 intracellular proteins that negatively modulate signaling pathways of G protein-coupled receptors (GPCRs).<sup>1–4</sup> GPCRs are a large family of plasma membrane receptors for endogenous neurotransmitters that couple to intracellular heterotrimeric GTP binding proteins (G proteins) comprising an  $\alpha$  subunit and a heterodimer of  $\beta\gamma$  subunits, which activate signaling cascades within the cell. GPCRs are responsible for the actions of many different addictive substances either as direct targets or as a secondary consequence of the action of the addictive agent. These receptors and their cognate signaling pathways not only mediate acute effects of addictive drugs/behaviors but are also vital to the development of the addictive disease whether this be addiction to legal (alcohol, nicotine) or illegal (amphetamine, cocaine, heroin, cannabis) drugs, substances (solvents), or behavioral addictions (gambling). Because RGS proteins modulate GPCR signaling, they are important components in the control of intracellular events that

drive the addictive process. Indeed, the interaction between addictive drugs and RGS proteins appears to be interdependent because there is considerable evidence that RGS proteins are in turn manipulated by the addictive process.<sup>5</sup> Although it can be hypothesized that RGS proteins play a role in all addictions, published literature to date has concentrated on the stimulants amphetamine and cocaine and the opioid morphine.

**RGS protein modulation of G protein signaling**

Members of the GPCR family of receptors include the opioid receptors, especially the  $\mu$ -opioid receptor as the primary target for heroin and prescription opioids, the cannabinoid CB1 receptors, the dopamine D1 and D2 families of receptors that are the mediators of reward or the anticipation of reward for many addictions<sup>6,7</sup> as well as metabotropic glutamate receptors that may regulate many behavioral actions of addictive drugs.<sup>8</sup>

GPCRs show specificity for coupling to a particular type of  $G\alpha$  protein.  $G\alpha$  proteins are



**Figure 1.** G protein cycle. In the resting state the seven-transmembrane domain receptor is associated with the  $G\alpha\beta\gamma$  heterotrimeric G protein with the  $G\alpha$  subunit bound to GDP. Activation of the receptor promotes the exchange of GDP for GTP on the  $G\alpha$  subunit. This leads to separation of the  $G\alpha$  and  $G\beta\gamma$  subunits and allows them to interact with downstream effectors, such as adenylyl cyclase (AC) and ion channels. The pool of active  $G\alpha$ -GTP is slowly hydrolyzed by the GTPase activity of the  $G\alpha$  subunit. This process is accelerated by the GAP activity of RGS proteins. The inactive  $G\alpha$ -GDP has high affinity for the  $G\beta\gamma$  subunits, the heterotrimer is reformed, and the cycle can start again. By reducing the lifetime of the active  $G\alpha$ -GTP, RGS proteins also reduce the accumulation of the  $G\beta\gamma$  subunits and so negatively modulate all branches of G protein-mediated signaling.

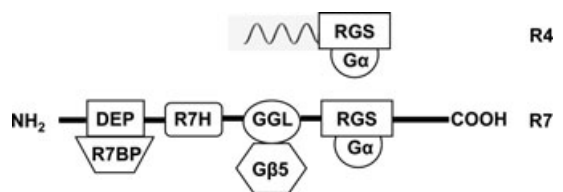
divided into four broad families:  $G\alpha_s$  activates the enzyme adenylyl cyclase leading to an increase in cAMP in the cell, the  $G\alpha_i/o$  family inhibits adenylyl cyclase, the  $G\alpha_q$  family activates the enzyme phospholipase C, leading to the release of calcium from intracellular stores, and  $G\alpha_{12/13}$  regulates cell growth and differentiation. The associated  $\beta\gamma$  subunits also interact with a variety of intracellular effectors including inwardly rectifying potassium channels, calcium channels, phospholipase C, and the mitogen-activated protein kinase pathway. Dopamine D1 receptors primarily couple to  $G\alpha_s$ , the opioid, cannabinoid, dopamine D2 receptors, and metabotropic glutamate receptors 2 and 3 predominantly couple to  $G\alpha_i/o$  proteins. In the resting state, the  $G\alpha$  of the heterotrimer G protein binds the guanine nucleotide GDP. Occupation of a GPCR by endogenous neurotransmitters or exogenous agonists activates the receptor, triggering the exchange of GDP for GTP on the  $G\alpha$  subunit and separation from the  $\beta\gamma$  subunits. Both  $G\alpha$ -GTP and  $\beta\gamma$  activate downstream effectors. Hydrolysis of the bound

GTP by the GTPase activity of the  $\alpha$  subunit provides  $G\alpha$ -GDP, which re-associates with the  $\beta\gamma$  heterodimers thus terminating the signal. RGS proteins bind to the GTP-bound  $G\alpha$  subunit and accelerate the rate of hydrolysis of GTP (Fig. 1).

RGS proteins are characterized by the presence of a consensus 125 amino-acid sequence, the so-called RGS box or RH homology domain (Fig. 2), which binds  $G\alpha$ -GTP proteins<sup>1-4,9</sup> and functions as a GAP (GTPase-accelerating protein).  $G\alpha_i/o$  and  $G\alpha_q$  subtypes of  $G\alpha$  proteins are sensitive to this GAP activity due to their relatively slow endogenous rate of GTP hydrolysis. In contrast,  $G\alpha_s$ , which already hydrolyses GTP rapidly, is insensitive to GAP activity. GAP action at GTP-bound  $G\alpha_i/o$ - or  $G\alpha_q$ -coupled receptors leads to a more rapid return to the inactive  $G\alpha$ -GDP state and a faster recombination of the  $G\alpha$  and  $\beta\gamma$  signaling molecules. By reducing the lifetime of  $G\alpha$ -GTP and  $\beta\gamma$  molecules, RGS proteins limit G protein signal strength at steady state and thus act as negative modulators of G protein signaling. As a result, RGS proteins exhibit profound control over both the potency and efficacy of agonist action. For example, in an *in vitro* system completely lacking RGS activity at  $G\alpha_o$ , the efficacy of morphine is increased several-fold and the potency 40-fold converting morphine from a partial agonist to a robust full agonist.<sup>10</sup>

## RGS protein families

Many proteins with RH domains have been identified. These proteins have been divided into various families (Table 1) on the basis of similarity in amino acid composition.<sup>2,4</sup> Members of the R4 and RZ families are smaller proteins consisting largely of



**Figure 2.** Cartoon of R4- and R7-type RGS proteins bound to  $G\alpha$ . Family R4 (e.g., RGS4) is essentially an RGS domain plus an N-terminal helical domain. The more complex R7 family (e.g., RGS9-2) contains protein-protein binding domains to allow association with other proteins, including the obligate partners R7 binding protein (R7BP) and  $G\beta_5$ . For other abbreviations see Table 1.

**Table 1.** Families of RGS proteins<sup>a</sup>

Family	Members	Structure: RGS domain plus
RZ	RGS17, 19, 20 <sup>b</sup>	N-terminal cysteine string
R4	RGS1, 2, 3, <sup>c</sup> 4, 5, 8, 13, 16, 18, 21	N-terminal amphipathic helix
R7	RGS6, 7, 9, 11	GGL + DEP + R7H domains
R12	RGS10, 12, 14	Variable—can contain PDZ RBD, and GoLoco domains
RA	Axin, Conductin	GSK + CAT + PP2A + DIX
RL	P115RhoGEF	DH + PH
RL	GRK2 and 3	Ser/Thr kinase + PH

<sup>a</sup>CAT,  $\beta$ -catenin binding site; DEP, disheveled, EGL-10, pleckstrin homology domain; DH, Dbl-homology (RhoGEF) domain; GEF, guanine-nucleotide exchange factor; GGL, G protein  $\gamma$ -like domain; GoLoco,  $G\alpha_{i/o}$ -GDP binding motif; GRK, G protein receptor kinase; GSK, glycogen synthase kinase  $3\beta$ -binding domain; PDZ, PSD95/Dlg/ZO-1 domain; PH, pleckstrin homology domain; R7H, R7 homology domain; PP2A, protein phosphatase 2A homology region; RBD, Rap1/2-binding domain; DIX, dimerization domain.

<sup>b</sup>RGS17 and 20 are also known as RGSZ2 and RGSZ1, respectively; RGS20 is also known as GAIP ( $G\alpha$ -interacting protein).

<sup>c</sup>RGS3 is much larger than other members of the R4 family and has multiple splice variants, some of which include a PDZ domain.

the RGS domain (Fig. 2). Other families have much more complex structures with domains that allow for protein-protein interactions. Particularly pertinent to addiction is RGS9-2 (Fig. 2), a member of the R7 family, which, in addition to the conserved RGS box, contains several other domains/motifs. R7 family members possess a DEP (disheveled, Egl-10, pleckstrin) domain that binds the adapter protein R7BP,<sup>11,12</sup> which promotes membrane association<sup>13,14</sup> and stabilizes RGS9-2 against degradation. The R7 family members also have a GGL (G gamma like) domain that allows binding to the G protein  $\beta 5$  subunit ( $G\beta 5$ ),<sup>15</sup> which further promotes protein stability.<sup>16</sup> RGS proteins may also be regulated by phosphorylation<sup>17,18</sup> and lipid modification.<sup>19</sup> Strongly acidic lipids, such as phosphatidyl inositol trisphosphate ( $PIP_3$ ) and phosphatidic acid, transiently bind to and inhibit the GAP activity of R4 family members; an inhibition that can be reversed by calcium/calmodulin.<sup>20,21</sup>

In addition to RGS proteins that act as GAPs, there are other proteins with RH domains that bind to  $G\alpha$  but do not necessarily exhibit GAP activity. The RH domain of GPCR kinases (GRKs) that are recruited by  $\beta\gamma$  subunits for receptor phosphorylation binds to  $G\alpha q$  and inhibits its ability to activate phospholipase C.<sup>22</sup> The Rho guanine nucleotide exchange factors (RhoGEFs) bind to the G proteins  $G\alpha_{12}$  and

$G\alpha_{13}$ <sup>23</sup> and also interact with  $G\alpha q$ .<sup>24</sup> The primary function of the RH domain in the RH-RhoGEF proteins is to link the GPCR to Rho-mediated signaling pathways that control many cell processes including the actin cytoskeleton, cell growth, proliferation, and differentiation.<sup>25</sup> The proteins axin and conductin regulate the Wnt signaling pathway important for embryogenesis and cancer.

Although many RGS proteins are GAPs for all members of the  $G\alpha_{i/o}$  and  $G\alpha q$  families, some exhibit selectivity. For example members of the R7 family, which includes RGS9-2, are selective for  $G\alpha_o$ ,<sup>26,27</sup> whereas RGS2 is considered to be more effective as a GAP at  $G\alpha q$ .<sup>28</sup> There is also increasing evidence that specificity for RGS proteins can be conferred by the GPCR.<sup>29–32</sup> Several RGS proteins, including RGS2, 4, 8, and 9, have been shown to act as GAPs to modulate  $\mu$ -opioid receptor signaling *in vitro*, but there may be specificity under endogenous conditions.<sup>33</sup> However, the distribution of RGS protein types relative to receptor and  $G\alpha$  subtype distribution may be the most important facet governing selectivity.<sup>5</sup>

### Distribution of RGS proteins

mRNA for the various members of the RGS protein family is unevenly distributed across the central

nervous system. In particular, there is specific localization of certain RGS proteins in regions implicated in the action of addictive drugs. RGS2 mRNA is found in the periform and neocortex, caudate putamen, amygdala, hippocampus, and locus coeruleus (LC).<sup>34,35</sup> RGS4 mRNA is found in high levels in the thalamus, caudate putamen, and nucleus accumbens (NAcc), as well as the prefrontal cortex,<sup>36,37</sup> where incorrect dopamine and serotonin function may be responsible for some of the symptoms of schizophrenia. Indeed, RGS4 has been suggested as a susceptibility gene for schizophrenia<sup>38,39</sup> and RGS4 protein is reduced in the prefrontal cortex of human schizophrenic post mortem brains<sup>40</sup> and in a rat model of schizophrenia.<sup>41</sup>

RGS9 is found in two forms: a short form (RGS9-1) that is exclusive to the retina, where it acts as a GAP for the retinal G protein  $G_{\alpha t}$ , and a longer form (RGS9-2) that is largely confined to striatal regions in rodent and human brain, that is, the caudate putamen, NAcc, islands of Calleja, and olfactory tubercle.<sup>36,42-44</sup> This distribution mimics that of striatal specific proteins, such as dopamine D1 receptors, adenylyl cyclase type V, and the phosphoprotein DARPP-32 (dopamine and cyclic adenosine 3',5'-monophosphate regulated phosphoprotein with molecular mass of 32 kDa).<sup>45</sup> In addition, RGS9-2 is expressed at lower levels in regions associated with the antinociceptive actions of morphine, namely the periaqueductal gray (PAG) and the dorsal horn of the spinal cord.<sup>46</sup>

### Interactions between RGS proteins and drugs of abuse

The localization of RGS2 and 4 and in particular RGS9-2 led to speculation of a role in addictive processes. This idea is strengthened by several reports showing that addictive substances cause changes in the levels of RGS proteins or RGS mRNA, suggesting a role for these drug-induced modifications in the addictive process. Several studies have addressed changes in RGS after drug exposure but there have been fewer studies on the roles of RGS proteins in modulating responses to addictive drugs.

### RGS2

The mRNA for RGS2 has a short half-life,<sup>47</sup> and the RGS2 protein itself is targeted for proteasomal

degradation,<sup>48</sup> suggesting that RGS2 protein levels are tightly regulated. Although no studies to date have demonstrated changes in RGS2 protein in response to addictive drugs, there is evidence for alterations at the mRNA level. In the rat, administration of amphetamine, methamphetamine, or cocaine rapidly upregulates RGS2 mRNA levels in the striatum<sup>47</sup> on a rapid time scale similar to the induction of *c-Fos* expression.<sup>49</sup> Moreover, the upregulation is transient and persists with repeated drug administration,<sup>50</sup> indicating that RGS2 may be involved in the acute actions of amphetamine. Also, because rapid alterations in RGS2 mRNA levels occur repeatedly with each amphetamine administration, RGS2 may function as a modulator for neuroadaptive changes that lead to dependence and sensitization.<sup>50</sup>

Increases in synaptic dopamine after administration of stimulants probably underlie the rapid changes in RGS2. Both the rat and human gene for RGS2 contain cAMP-sensitive elements<sup>51,52</sup> (the human gene has a CRE<sup>51</sup>) and so dopamine stimulation of adenylyl cyclase via  $G_{\alpha s}$ -coupled D1 receptors will lead to an increase in RGS2 message. Indeed, dopamine D1 antagonists prevent the amphetamine-induced upregulation of RGS2 mRNA.<sup>53</sup> Conversely, activation of the  $G_{\alpha i/o}$ -coupled D2 receptor inhibits adenylyl cyclase, leading to decreased RGS2 mRNA expression, whereas D2 antagonists, such as haloperidol, stimulate RGS2 mRNA expression and show an additive effect with amphetamine.<sup>47,53</sup>

The rapid and selective upregulation of RGS2 mRNA in response to stimulants indicates a role for RGS2 in the control of dopamine-mediated signaling events. Although RGS2 preferentially acts at  $G_{\alpha q}$  it can also act as a GAP to regulate  $G_{\alpha o}$ .<sup>54</sup> Consequently, increased levels of RGS2 would probably reduce D2 receptor signaling by this GAP activity. Yet, there is no similar GAP activity at the  $G_{\alpha s}$ -coupled dopamine D1 receptor. Therefore, the ability of RGS2 to modulate D2 but not D1 signaling could explain the altered balance between D1 and D2 signaling that is thought to underlie adaptive processes to chronic cocaine. On the other hand, RGS2 as well as several other RGS proteins (RGS3, 4, 10, and 13) directly inhibit adenylyl cyclases 2, 5, and 6.<sup>55,56</sup> The mechanism behind this effect is unknown but could be due to a direct interaction of RGS2 with  $G_{\alpha s}$ , leading to GAP-independent

inhibition, and/or a direct interaction with the adenylyl cyclase enzyme.<sup>57</sup> Thus, although RGS2 does not directly modulate D1 receptor signaling by acting as a GAP, it might negatively regulate D1 receptor signaling to adenylyl cyclase.

The rapid control of RGS2 expression by stimulants provides a potential feedback mechanism to finely tune dopaminergic signaling. By acting at an early stage of the signaling cascade, RGS2 can inhibit multiple branches of the D2 receptor signaling cascade as well as D1 receptor signaling to adenylyl cyclase and its downstream effects. In cases where both receptors are coexpressed on the same neuron there is the potential for cross-talk. Consequently, RGS2 can regulate many dopamine-induced behaviors associated with drugs of abuse.

Chronic morphine, acting at  $G\alpha i/o$ -coupled  $\mu$ -opioid receptors to inhibit adenylyl cyclase, has been reported to reduce RGS2 mRNA in dopaminergic neurons in the ventral tegmental area (VTA) of mice.<sup>58</sup> In contrast, precipitation of opioid withdrawal leads to a modest increase in RGS2 message in the LC.<sup>59</sup> Chronic administration of the "date rape" drug GHB ( $\gamma$ -hydroxybutyrate), acting at  $GABA_B$  receptors similarly reduces RGS2 mRNA expression in the VTA,<sup>58</sup> a region wherein  $GABA_B$  receptor coupling to inwardly rectifying potassium channels is sensitive to modulation by RGS2. Therefore, the GHB-induced reduction in RGS2 mRNA might provide positive feedback to enhance GABA coupling efficiency and increase the potency of GHB to inhibit dopamine firing rates. Indeed, GHB-induced decreases in RGS2 mRNA are accompanied by a loss of drinking preference for GHB.<sup>58</sup>

## RGS4

RGS4 is also an unstable protein that is subjected to N-end rule degradation<sup>48,60</sup> and is regulated by transcription and RNA stabilization.<sup>61</sup> Addictive drugs generally lead to increased RGS4 mRNA expression, although the effects are not as marked as with RGS2 or as consistent,<sup>53,59,62–64</sup> which may be due to region and temporally specific changes. In the severely morphine-dependent mouse there is a downregulation of RGS4 mRNA in the lateral hypothalamus.<sup>64</sup> In the rat, RGS4 protein is upregulated in the NAcc after acute morphine but downregulated in the LC,<sup>63</sup> where RGS4 protein is expressed mainly in nora-

adrenergic (tyrosine hydroxylase positive) neurons.<sup>59</sup> In contrast, treatment of rats with high levels of morphine for several days led to a twofold increase in the level of RGS4 protein in the LC, but with no change in the mRNA for RGS4.<sup>59</sup> The increased protein expression returned to baseline after precipitation of withdrawal with the opioid antagonist naloxone, accompanied by a threefold increase in RGS4 message. A plausible rationale for this seeming mismatch between RNA and protein is that the protein is stabilized in the presence of chronic morphine such that withdrawal of morphine leads to increase proteolytic breakdown, as a consequence of which the level of mRNA is increased.<sup>59</sup> Although large doses of morphine and naloxone were used in these studies, no changes were observed in the expression of several other RGS proteins and no changes in RGS4 protein or message were observed in the PAG, an area important for morphine antinociception. On the other hand, RGS4 mRNA was decreased in the occipital lobe after precipitated withdrawal, an effect that was attributed to withdrawal-induced increases in noradrenergic signaling at  $G\alpha s$ -coupled stimulatory  $\beta$ -adrenergic receptors.<sup>59</sup>

*In vitro*, RGS4 negatively modulates signaling at opioid receptors by acting as a GAP.<sup>59,65</sup> Therefore, the increased RGS4 seen in the LC after chronic morphine exposure will reduce  $Gi/o$ -mediated signaling further and so contribute to tolerance development. However, there are conflicting reports from studies using RGS4-knockout mice of the importance of RGS4. A study of RGS4-knockout mice has reported no morphine phenotype,<sup>66</sup> which might indicate that other RGS proteins are functionally redundant with RGS4. In contrast, a study with an independent strain of mice has reported increased reward and more severe withdrawal symptoms.<sup>67</sup>

The response of RGS4 mRNA levels to stimulants has been various and assay dependent.<sup>50,54,63,68–71</sup> Yuferov *et al.*<sup>69</sup> reported a decrease in RGS4 mRNA in the caudate putamen of Fisher rats after 3 days of binge cocaine. Similarly, Schwendt *et al.*<sup>71</sup> report a decrease in RGS4 mRNA expression in the prefrontal cortex and dorsolateral striatum 21 days after abstinence from cocaine either self-administered or by noncontingent binge pattern administration. Strikingly, exposure of the rats to the cocaine-associated environment caused a return of RGS4 to normal levels, indicating a role for RGS4 in the long-term actions of cocaine.

Dopamine receptor agonists control RGS4 mRNA levels in a manner that is opposite to that of RGS2.<sup>53,68</sup> Dopamine D1 agonists decrease RGS4 gene expression and D2 agonists increase RGS4 gene expression. Consequently, observed changes in RGS4 may result from the altered balance of dopamine receptor functioning that occurs after prolonged cocaine where there is an increase in D1 function and a decrease in D2 function. Alternatively, blockade of *N*-methyl-D-aspartate receptors decreases RGS4 mRNA,<sup>72</sup> suggesting a role for the cocaine-induced downregulation of *N*-methyl-D-aspartate receptor subunits. With the potential negative modulation by RGS4 of G $\alpha$ i/o-coupled D2 receptors, the reduction in RGS4 may contribute to resetting the balance of dopamine D2 and D1 signaling.

## RGS9-2

Changes in RGS9-2 protein levels after morphine have been reported, but the direction is temporally dependent. Two hours after acute morphine, RGS9-2 protein levels increase by 50% in the caudate putamen and NAcc of mice, along with regions involved in the antinociceptive actions of morphine, namely, the PAG and dorsal horn of spinal cord.<sup>46,73</sup> In contrast, chronic exposure to morphine reverses this rise and ultimately leads to a 50% reduction in basal RGS9-2 protein levels. No changes in the levels of the binding partner G $\beta$ 5 were observed.<sup>73</sup>

These dynamic changes in RGS9-2 protein seen after morphine administration are not accompanied by alterations in mRNA levels. Therefore, morphine treatment might alter the protein itself, as appears to occur with the smaller R4 family members. This may result from the complex structure of RGS9-2 and its requirement for binding partners, such as G $\beta$ 5 and R7BP, which confer protein stability and localization as well as promote proper folding of RGS9-2.<sup>13–16</sup> Interruption or stabilization of these protein binding and folding processes could lead to mRNA-independent changes in RGS9-2 levels. For example, members of the R7 family have a conserved Pro–Glu–Ser–Thr (PEST) sequence in the GGL domain, a signal for protein degradation that may be hidden by the binding of G $\beta$ 5.<sup>74</sup>

The physiological roles of RGS9-2 protein in the actions of abused drugs have been inferred from studies using targeted gene deletion or antisense

oligonucleotides to knock out/knock down RGS9-2 in rodents. RGS9-knockout mice showed more potent responses to morphine related to their abuse potential and other pharmacologies.<sup>46</sup> For example, in the conditioned place preference assay mice are trained to associate administration of drug with a particular environment as an indication of reward. In this assay morphine is “rewarding” at low doses, although this effect is reversed at higher doses. RGS9-null mice show an approximately 10-fold higher potency of morphine in this assay than littermate wild-type controls. The increased potency of morphine is reversed by restoration of RGS9-2 protein levels by injection of virus expressing RGS directly into the in the NAcc.<sup>46</sup> In contrast, overexpression of RGS4 has no effect, suggesting a selectivity of RGS9-2. Because both RGS9-2 and RGS4 readily act as GAPs for G $\alpha$ o, this selectivity is likely to be achieved by the correct targeting of the more complex RGS9-2 to the  $\mu$ -opioid receptor.

In mice null for RGS9<sup>46</sup> or where RGS9 has been knocked down by antisense treatment,<sup>75,76</sup> there is an increase in morphine-mediated antinociception and a delay in the development of antinociceptive tolerance. Similarly, knockdown of the R7 family binding partner G $\beta$ 5 enhances the antinociceptive response to the  $\mu$ -opioid receptor agonist DAMGO and inhibits acute tolerance.<sup>76</sup> These findings suggest that endogenous RGS9-2 inhibits  $\mu$ -opioid-mediated antinociception. Thus, the increase in RGS9-2 expression after acute morphine might enhance this negative modulation and so contribute to the loss of effect, that is, tolerance. On the other hand, antisense knockdown of RGS9 and G $\beta$ 5 facilitates tolerance to more sustained morphine exposure.<sup>75,76</sup> The reduction in RGS9-2 after chronic morphine will therefore act to maintain antinociception as tolerance develops, although this could incur the cost of increased dependence. Indeed, compared to wild-type mice, RGS9-knockout mice show a significant increase in behaviors associated with withdrawal including jumping, wet-dog shakes, paw tremor, diarrhea, and ptosis, as well as an increased in *c-Fos* expression in the LC.<sup>46</sup> It appears from studies to date that RGS9-2 modulates all morphine pharmacology without selectivity.

In contrast to chronic morphine, chronic cocaine has been reported to cause relatively small increases in RGS9-2 protein in the NAcc and caudate

putamen, particularly in animals that self-administered the drug.<sup>77</sup> As observed with morphine there was no change at the mRNA level. In contrast, acute amphetamine has been reported to reduce RGS9 mRNA expression in the dorsal striatum of male rats,<sup>47</sup> although a second study using direct D1 and D2 agonists showed no effect.<sup>53</sup> Despite these small changes RGS9-2 does appear to be important for modulating dopamine signaling in striatal regions. RGS9-2 is expressed in dopamine D2 expressing neurons in the NAcc<sup>44</sup> and acts as a GAP only for G $\alpha$ o, which is almost exclusively responsible for D2 receptor signaling.<sup>78</sup> Moreover, mice lacking RGS9 show a dopaminergic-mediated dyskinesia,<sup>79</sup> and MTPT (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated monkeys show a dyskinesia that is significantly reduced by viral expression of RGS9 in the dorsal striatum.<sup>80</sup> Also, monkeys overexpressing RGS9 exhibit a reduced susceptibility to L-dopa-induced dyskinesias.<sup>80</sup>

As a consequence of loss of negative modulation of D2 signaling, RGS9-knockout mice demonstrate increases in cocaine-induced locomotor activity, which can be prevented by overexpression of RGS9-2 in the NAcc.<sup>74</sup> In addition, these mice show a potentiated locomotor sensitization on repeated cocaine administration; sensitization of stimulant-mediated behaviors is considered a major component of drug-seeking behavior. The rewarding effect of cocaine (as measured by place preference) is also increased, although the effect is not as profound as with morphine.

Exposure of ovariectomized female rats to estrogen for 2 weeks has been reported to cause a 30% decrease in the level of mRNA for RGS9 in the shell of the NAcc.<sup>81</sup> A resulting reduction in RGS9-2 protein should lead to increased signaling at G $\alpha$ i/o-coupled receptors. Such a mechanism might explain the increase in dopamine function caused by estrogen and consequently may contribute to the increased vulnerability to the reinforcing effects of drugs of abuse seen in females.<sup>82</sup>

### Other RGS proteins

There are isolated reports linking RGS proteins other than RGS2, 4, and 9 to abused drugs. Several of these results are from microarray analysis profiling after alcohol or nicotine in addition to changes induced by opioids or stimulants.

### R4 family

RGS3 and RGS5 are upregulated after amphetamine in the striatum of the rat, although this requires more sustained stimulation than required to upregulate RGS2.<sup>47</sup> However, as with RGS2, upregulation of RGS3, but not RGS5, persists with repeated amphetamine administration.<sup>50</sup> A reduction in RGS13 has been reported in the prefrontal cortex, NAcc, VTA, and amygdala of rats given chronic nicotine.<sup>83</sup> Nicotine challenge after intermittent exposure and withdrawal resulted in decreased expression of RGS16 in several mouse strains but an increase in a quasi-congenic RQI (recombinant quantitative trait loci introgression) strain.<sup>84</sup>

### R7 family

A small increase in RGS7 protein, after acute, but not chronic, morphine is seen in the LC.<sup>59</sup> As with knockdown of RGS9, antisense knockdown of other R7 family members RGS6, 7, or 11 gives an increase in morphine-mediated antinociception and a delay in the development of antinociceptive tolerance.<sup>76</sup> In the C3H/HeJ mouse, but not the C57/BL6 mouse, there is a significant regulation of RGS11 in the amygdala and hippocampus in response to chronic nicotine.<sup>85</sup>

### RZ family

RGS20 has been shown to modulate  $\mu$ -opioid receptor signaling *in vitro*,<sup>86</sup> and antisense studies have demonstrated that both RGS19 and RGS20 enhance morphine antinociception in the mouse.<sup>87</sup> Withdrawal from chronic ethanol in mice showed a downregulation of RGS19-interacting protein, a protein that binds to both RGS19 and G $\alpha$  and targets G $\alpha$  for degradation.<sup>88</sup>

## RGS proteins as potential targets for the treatment of addictive diseases

Levels of several RGS proteins are manipulated by addictive drugs, and experiments with knock-down/knockout of RGS proteins show that several of these proteins modulate signaling pathways downstream of GPCRs involved in addiction and subsequent behaviors. With the fine-tuning that this interdependency can evoke, it is possible to envisage RGS proteins might be targets for drug abuse treatment. Obviously the study of the role of RGS proteins in addiction would benefit from the

development of small-molecule inhibitors of RGS proteins. This research is still in its infancy, although there are indications for interaction sites at the RGS–G $\alpha$  interface that could be specific for particular RGS–G $\alpha$  combinations<sup>89</sup> and that compounds that target G $\alpha$ –RGS interactions can be found, either by high-throughput screening<sup>90–92</sup> or rational design.<sup>93</sup>

An immediate target for intervention, with its distribution and ability to modulate both morphine-induced and cocaine-induced behaviors, is RGS9-2. On the basis of studies in rodents, a compound that stimulates RGS9-2 levels or activity would be expected to decrease stimulant and opioid reward as well as dependence and drug-seeking behavior. Conversely, in the pain clinic, where enhanced analgesic activity of morphine is required, an inhibitor of RGS9-2 activity would be the required target, predicted to enhance the antinociceptive response to morphine and decrease acute tolerance. However, because RGS9-2 appears to modulate all  $\mu$ -opioid-mediated behaviors, such an inhibitor would also increase dependence, reward, and other unwanted actions. An alternative approach might be to use an RGS9-2 inhibitor as an adjunct to a partial agonist (such as buprenorphine) where additional selectivity of action could be obtained based on efficacy.<sup>94,95</sup>

The small RGS proteins, such as RGS2 and RGS4, are less likely targets. One RGS4-knockout mouse strain shows no opioid phenotype, indicating that there may be functional redundancy for GAP activity. Moreover, RGS2 and RGS4 are widely expressed throughout the brain and thus much less likely to provide a selective target, although it is possible that selectivity could be imposed through specific G $\alpha$  subtype and receptor interactions. Furthermore, RGS4 is important in the parasympathetic control of heart rate,<sup>96</sup> whereas RGS2 is important for blood pressure regulation.<sup>97</sup> Interference with these proteins is likely to have serious cardiovascular effects.

Of the more than 30 RGS proteins identified, relatively few have been studied in detail, yet several have been reported to show changes in expression in response to addictive drugs and may be important in specific aspects of the pharmacology of addiction. To study each RGS protein individually is a daunting task. An alternative approach is to use systems that express G $\alpha$  proteins with a mutation in the conserved RGS binding domain to render them RGS insensitive.<sup>98</sup> Such systems allow the study of

global RGS protein inhibition on receptor signaling. Mice expressing these RGS-insensitive G $\alpha$  proteins are now available.<sup>99</sup> Such mice will probably show altered pharmacology across a whole spectrum of effects but will at least provide proof of principle that the pharmacology of addictive drugs can be profoundly altered by inhibition of RGS protein GAP activity. We can look forward to learning much more about the roles of these important accessory proteins in the pharmacology of addiction and as possible targets for addictive medications.

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## Conflicts of interest

The author declares no conflicts of interest.

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