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Addictive drugs modulate GIRK-channel signaling by regulating RGS proteins

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Regulator of G-protein signaling (RGS) proteins are strong modulators of G-protein-mediated pathways in the nervous system. One function of RGS proteins is to accelerate the activation–deactivation kinetics of G-protein-coupled inwardly rectifying potassium (GIRK) channels. The opening of GIRK channels reduces the firing rates of neurons. Recent studies indicate that RGS proteins also modulate the coupling efficiency between γ-aminobutyric acid type B ($GABA_B$) receptors and GIRK channels in dopamine neurons of the ventral tegmental area (VTA), the initial target for addictive drugs in the brain reward pathway. Chronic drug exposure can dynamically regulate the expression levels of RGS. Functional and behavioral studies now reveal that levels of RGS2 protein, through selective association with GIRK3, critically determine whether $GABA_B$ agonists are excitatory or inhibitory in the VTA. The regulation of RGS protein in the reward pathway might underlie adaptation to different types of addictive drugs.

Introduction

The brain contains an intrinsic reward system that originates in the ventral tegmental area (VTA) and is activated by unexpected natural rewards, such as food and sex, and addictive drugs (Box 1). Within the VTA, dopamine (DA) neuron activity is controlled, in part, by γ-aminobutyric acid (GABA) interneurons. Recently, we have shown that γ-hydroxybutyrate (GHB), an addictive club drug, can activate DA neurons through its action on $GABA_B$ receptors [1,2]. $GABA_B$ receptors are members of a large family of G-protein-coupled receptors (GPCRs), which contain seven transmembrane domains and signal via heterotrimeric G proteins (Gαβγ). Activation of GPCRs promotes the exchange of GTP for GDP on the Gα subunit. Activated G proteins then dissociate into Gα–GTP and Gβγ dimers, which interact with a wide-range of effectors, including cyclases, lipases and ion channels. The Gα subunit terminates the activation by hydrolyzing GTP into GDP and reassembling the inactive heterotrimer ($G\alpha_{\text{-GDP}}$–Gβγ).

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Activation of $GABA_B$ receptors leads to opening of G-protein-coupled inwardly rectifying potassium (GIRK) channels. The most abundant GIRK subunits in the mammalian brain are GIRK1, GIRK2 and GIRK3, which assemble into homotetramers (GIRK2) or heterotetramers (GIRK1/2, GIRK1/3, and GIRK2/3) [3]. GIRK channels preferentially enable K^+ ions to enter the neuron (referred to as inward rectification). However, the small outward flow of K^+ ions is of physiological relevance because it reduces the excitability of neurons. Stimulation of GPCRs that communicate through pertussis toxin (PTX)-sensitive G proteins (the $G_{i/o}$ family), such as the $GABA_B$ receptor, activates GIRK channels through the direct binding of G-protein Gβγ dimers to the channel [3]. More recent evidence indicates that the PTX-sensitive Gα subunits also associate directly with the channel, indicating that the inactive heterotrimer is situated near to the channel forming a signaling complex [4,5] (Figure 1). In summary, GIRK channels contribute to the resting membrane potential of neurons and, upon receptor stimulation, generate a hyperpolarizing postsynaptic potential [3].

Changing the strength of GPCR signaling in the VTA might affect the response to addictive drugs. In principle, there are three distinct mechanisms for modifying GPCR signaling. One way is through GPCR desensitization, which involves both clathrin-mediated endocytosis [6] and uncoupling of G proteins [7]. Another mechanism is through changes in effector activity, such as downregulation of GIRK and calcium channels [5,8]. Lastly, changes in G-protein availability can alter GPCR signaling. G-protein availability can be influenced by guanine-nucleotide-dissociation inhibitor proteins (GDI) [9] and by regulator of G-protein signaling (RGS) proteins. The family of RGS proteins contains a GTPase-activating protein (GAP) domain that promotes the formation of the inactive G-protein heterotrimer [10,11]. In addition to the GAP domain, different RGS proteins contain a wide range of other signaling domains [12]. RGS proteins have received much attention as key proteins in the response to addictive drugs [13]. The functional consequences of changes in RGS proteins, however, are not well understood.

We have recently discovered that regulation of a RGS protein subtype in the brain reward pathway (Box 1)

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Box 1. The mesocorticolimbic dopamine system in health and disease

The mesocorticolimbic dopamine (DA) system originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), the prefrontal cortex, the septum, the amygdala and the hippocampus. The majority of projection neurons release the neuromodulator DA when the basal firing activity converts to burst firing. Under physiological conditions, this burst-firing of DA neurons occurs when a reward is received by surprise. If a conditioned stimulus (CS) predicts the reward, burst-firing activation occurs with the CS instead of the now expected reward. If, by contrast, a CS is presented and no reward delivered, DA neurons reduce firing. Taken together, one function of DA neurons is to code for the prediction error of reward rather than reward itself [47].

All addictive drugs increase DA concentrations in target nuclei of the mesocorticolimbic system. A leading hypothesis to explain addiction is that the release of DA, even when reward is expected, generates a pathological learning signal that represents the first step towards compulsion [48]. At the cellular level, inappropriate release of DA might trigger adaptive phenomena, such as drug-evoked synaptic plasticity in the VTA and its target nuclei.

The molecular determinants underlying the increase of DA levels are specific for each class of drug, but occur through three main cellular mechanisms [32]. Opioids, cannabinoids, GHB and, probably, benzodiazepines primarily decrease the activity of GABA interneurons, leading to disinhibition of DA neurons. Nicotine directly depolarizes DA neurons. Psychostimulants, such as amphetamines, cocaine and ecstasy, interfere with DA uptake, leading to elevated levels of synaptic DA.

Addictive drugs also induce dependence, which, in contrast to addiction, is defined by the occurrence of a withdrawal syndrome upon abrupt termination of drug exposure. Dependence is typically associated with tolerance, which requires that subjects increase the dose to obtain the same drug effect. The observation that chronic GHB exposure makes acute administration of GHB inhibit, rather than excite, DA neurons, might reflect a special form of tolerance that cannot be overcome by increasing the GHB dose.

dramatically alters GIRK-channel signaling in DA neurons [2]. Here, we discuss the evidence for the involvement of RGS proteins in addiction, the role of RGS2 proteins in directly modifying signaling through GIRK channels and postulate the role of RGS proteins in regulating GPCR signaling in other pathways.

An extended family of RGS proteins

RGS proteins comprise a large family of proteins that contains >37 members [12]. Each RGS protein has a conserved 120 amino acid core domain, commonly called the RGS domain, which is responsible for the GAP activity [14,15]. RGS proteins are widely expressed throughout the brain, where they can potentially modulate GPCR-mediated signaling [16]. Individual RGS proteins interact with particular Gα subunits, which might be determined by specific sequences in the RGS domain, their selective expression and the corresponding Gα subunit. For example, two RGS9 splice variants, RGS9–1 and RGS9–2, differ only in the C-terminal tail, which confers selectivity with interacting proteins [17]. In addition, cell-type-specific expression distinguishes the splice variants. RGS9–1 is observed almost exclusively in the retina and is responsible for acceleration of hydrolysis of GTP by Gαt. RGS9–2, in turn, is strongly expressed in the striatum and regulates DA D_2 and μ-opioid receptors coupled to $G\alpha_{i/o}$ [18]. Thus, the combination of cell-type-specific expression and selective protein–protein association for different RGS proteins indicates key functional roles for specific RGS proteins in the brain.

Using GIRK channels as ultra-sensitive detectors (nM sensitivity) for Gβγ subunits, the effect of RGS proteins on GPCR signaling can be easily observed. In heterologous cells expressing GIRK channels and a GPCR, RGS proteins accelerate both activation and deactivation rates up to 100-fold [19]. For example, RGS1, RGS2, RGS3, RGS4, RGS5 and RGS8 proteins accelerate both activation and deactivation kinetics of GIRK currents after stimulation of muscarinic M_2 or 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors [20,21]. The acceleration of deactivation kinetics agrees with the GAP activity in the RGS. The faster rate of activation with RGS, by contrast, is not well understood.

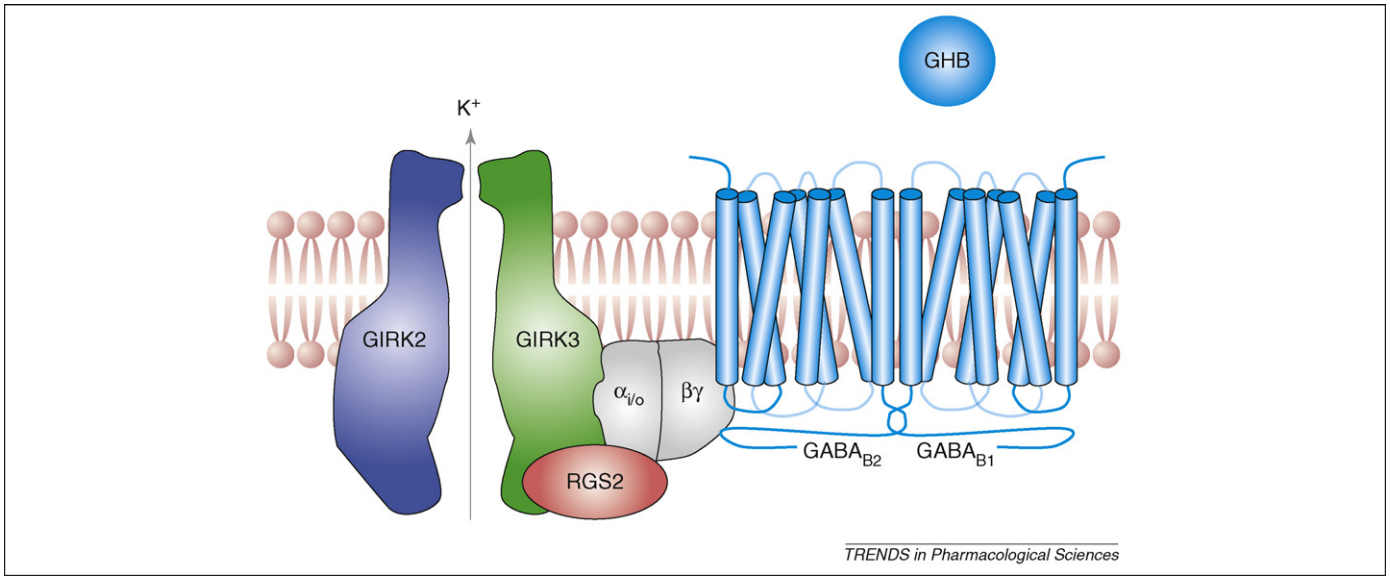

Figure 1. Macromolecular signaling complex in the resting state. A preassembled complex is postulated to exist between the dimeric $GABA_{B1}$ and $GABA_{B2}$ subunits, the heterotrimeric G proteins, GIRK2/3 channels and RGS2. Note that RGS2 interacts selectively with GIRK3 subunits and heterotrimeric G proteins associate directly with GIRK and $GABA_B$ receptors, thus enabling RGS to modulate the coupling efficiency (i.e. EC_{50}) between $GABA_B$ receptors and GIRK channels.

The GAP activity of the RGS would be expected to slow, not accelerate, the rate of activation. RGS proteins will increase the pool of available G proteins, which could accelerate activation [22]. Alternatively, for GIRK channels, the formation of a receptor–G-protein–GIRK complex has been proposed to promote faster activation [20,23,24]. Interestingly, both RGS7 and RGS8 accelerate the activation of GIRK current, but RGS8 more prominently accelerates deactivation [25]. These findings indicate that functional domains other than GAP could be involved in modulation.

RGS proteins: a member of the macromolecular signaling club

An emerging theme with GIRK signaling is that GPCRs, G proteins and GIRK channels exist in a macromolecular signaling complex [26] (Figure 1). Now, recent studies indicate that RGS proteins and GIRK channels might also interact directly within this complex. For example, the degradation-resistant RGS4 co-precipitates with several GPCRs and GIRKs forming stable macromolecular complexes, whereas RGS3s does not interact with the GPCR–GIRK channel complex [27]. These observations indicate a 'precoupling' model in the case of RGS4 versus a 'collision-coupling' model for RGS3. Precoupling can accelerate GIRK-channel gating with a 100-fold higher potency [27]. Further support for a macromolecular complex comes from fluorescence resonance energy-transfer (FRET) studies that demonstrate the interaction between RGS4 and $GABA_{B1}$ and $GABA_{B2}$ subunits [28], or between RGS2 and GIRK3 but not GIRK2 [2]. Thus, a subset of RGS proteins seem to interact selectively with GIRK channels.

Targeting RGS proteins to GIRK channels in a macromolecular complex might facilitate the RGS-mediated changes in the coupling efficiency between a GPCR and GIRK channel. Here, the coupling efficiency is determined by the EC_{50} concentration, that is, the agonist concentration necessary to activate 50% of the maximal GIRK current. Low coupling efficiency and, therefore, high EC_{50} reflect poor G-protein availability and low GIRK-channel affinity for Gβγ dimers. The value of the EC_{50} depends on several additional parameters, including receptor number, receptor–ligand affinity and agonist efficacy, which, however, remain constant under the conditions studied here.

Direct evidence that RGS proteins can specifically influence receptor–GIRK coupling comes from several studies [2,29,30]. First, co-transfection of RGS3s reduces the coupling efficiency (i.e. increases the EC_{50}) between muscarinic M_2 or $5HT_{1A}$ receptors and GIRK1/2 heteromeric channels *in vitro* [29,31]. Interestingly, the increase in EC_{50} was greater for RGS4 even though both RGS3s and RGS4 strongly accelerate the deactivation kinetics. Second, in acute slices of the VTA, selective expression of RGS2 in DA neurons modulates the coupling between $GABA_B$ receptors and GIRK channels [2]. Pharmacological inhibition of RGS proteins or genetic ablation of RGS2 in DA neurons decreases the EC_{50} from ~15 μM in control neurons to ~7 μM. Thus, the presence of RGS2 opposes G-protein activation of GIRK channels, leading to a higher EC_{50}. The lower Gβγ affinity for GIRK2/3 heteromeric channels also contributes to the shift in EC_{50} for baclofen activation [1,30] and DA neurons uniquely express GIRK2/3 channels. We observed a similar decrease in EC_{50} in GIRK3 knockout mice and RGS2/GIRK3 double-knockout mice [2]. These findings indicate that RGS2 specifically modulates the coupling to channels containing GIRK3. Indeed, a close association between GIRK3 and RGS2 is detected using FRET spectroscopic measurements [2]. Thus, specific protein–protein interactions among RGS proteins, G proteins, GIRK channels and $GABA_B$ receptors might be involved in establishing macromolecular signaling complexes that fine-tune the coupling efficiency of GPCR signaling.

Ups and downs of RGS expression

Owing to their role in modifying GPCR signaling, the expression of RGS proteins could be altered by chronic exposure to addictive drugs. Indeed, numerous studies

Table 1. Effects of addictive drugs on RGS expression[a]

Drug	Treatment	RGS mRNA	Brain region	Animal model	Refs
Morphine	Acute	↑ RGS9–2 protein	NAc[b], PAG and dorsal striatum	C57BL/6J mice	[39]
		↑ RGS9–2 protein	Striatum and thalamus	Albino mice CD-1	[40]
		↓ RGS9–2	Cortex	Albino mice CD-1	[40]
		↑ RGS4	NAc and DCG	Sprague-Dawley rats	[41]
		↓ RGS4	RtTg and LC	Sprague-Dawley rats	[41]
	Chronic	↓RGS9–2	NAc, PAG and dorsal striatum	Sprague-Dawley rats	[39]
		↑ RGS9–2	Striatum, thalamus, PAG and cortex	Albino mice CD-1	[40]
		↑ RGS4	LC	Sprague-Dawley rats	[41]
		↑ RGS4 protein	LC	Sprague-Dawley rats	[42]
		↓ RGS2	VTA (DA neurons)	C57BL/6J mice	[2]
GHB	Chronic	↓ RGS2	VTA (DA neurons)	C57BL/6J mice	[2]
Cocaine	Acute	↑ RGS4	NAc and DCG	Sprague-Dawley rats	[41]
		↓ RGS4	RtTg and LC	Sprague-Dawley rats	[41]
		↓RGS2	Hippocampus, cortex and striatum	Sprague-Dawley or Fischer-344 rats	[43]
	Chronic	↑RGS4	NAc and CPu	C57BL/6J mice	[44]
		↑RGS4	LC	Sprague-Dawley rats	[41]
Amphetamine or methamphetamine	Acute	↑ RGS2, RGS3 and RGS5	Striatum	Fischer rats	[45]
		↓ RGS4	Forebrain	Sprague-Dawley rats	[46]
		↑ RGS4	NAc and DCG	Sprague-Dawley rats	[41]
	Chronic	↑ RGS4	LC	Sprague-Dawley rats	[41]

[a]Compilation of studies reporting changes in RGS-mRNA expression levels with acute or chronic exposure to addictive drugs. For discussion, see text.
[b]Abbreviations: Cpu, caudate putamen; DCG, dorsal central gray; LC, locus coeruleus; NAc, nucleus accumbens; PAG, periaqueductal gray; RtTg, reticulo-tegmental nucleus.

have demonstrated that the expression levels of RGS genes and proteins in the brain are dynamically regulated with drugs of abuse [13]. Both elevations and reductions in RGS transcripts and protein have been described with exposure to addictive drugs, however, indicating an important, but poorly understood, adaptive function with chronic drug exposure. We consider two classes of addictive drugs [32] (Box 1): psychostimulants, such as cocaine and methamphetamine, which lead to elevated DA levels and prolonged DA signaling; and morphine and the club drug GHB, which activate opioid and $GABA_B$ receptors, respectively. Acute administration of cocaine and amphetamines reduces RGS in some regions of the brain, whereas increasing RGS in other regions (Table 1). Chronic exposure to psychostimulants usually increases levels of RGS transcripts. Acute administration of morphine typically increases RGS expression, whereas chronic exposure reduces RGS expression – the opposite response of psychostimulants. Exceptions to these observations, however, might reflect species differences or subtle variations in the treatment protocols. We found that chronic GHB treatment (injections twice-daily for one week) leads to a reduction of RGS2 mRNA in DA neurons of the VTA [2] (Figure 2). In summary, addictive drugs lead to bidirectional changes in the levels of RGS transcripts and protein, indicating complex changes in G-protein signaling, depending on the cell type and region of the brain.

Whereas it is clear that RGS levels can be up or downregulated in specific regions of the brain in response to drugs of abuse, the functional consequence of this change in expression has remained more elusive. Few studies have examined the behavioral consequences of changes in RGS protein levels. In one study, overexpression of RGS9–2 in the nucleus accumbens (NAc) reduced the locomotor responses to cocaine, whereas RGS9-deficient mice showed augmented locomotor and rewarding responses to cocaine [33]. Conversely, we found that chronic exposure to GHB decreases RGS2 protein [2] (Figure 2), which

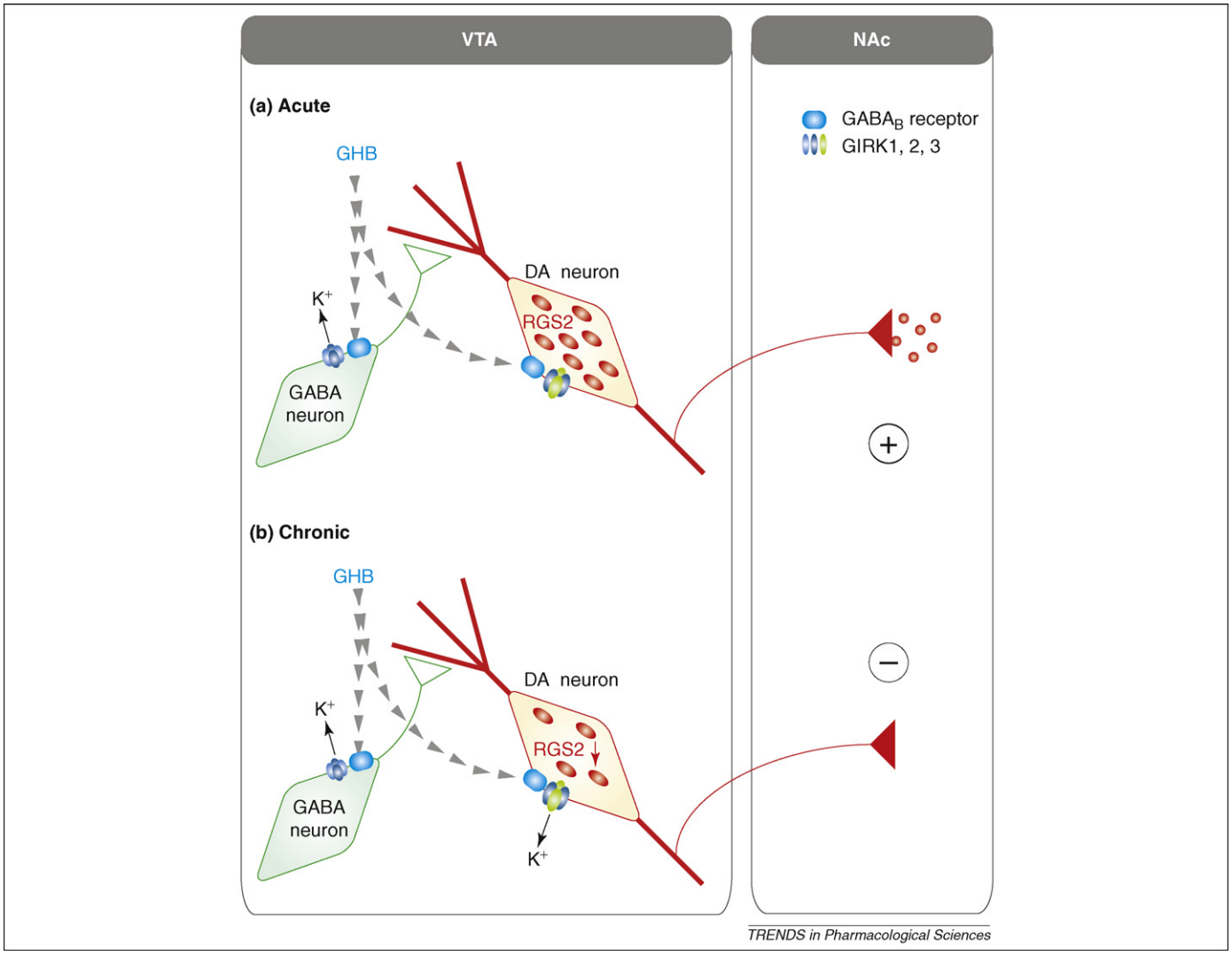

Figure 2. Chronic GHB-dependent decrease in RGS2 protein in VTA–DA neurons changes the neuronal excitability and behavioral response to GHB. Acute administration of GHB stimulates $GABA_B$ receptors **(a)** coupled to GIRK channels in GABA neurons (green). DA neurons (red) are unresponsive to GHB because high levels of RGS2 reduce the coupling efficiency (high EC_{50}) between $GABA_B$ receptors and GIRK channels. Opening of GIRK channels in GABA neurons disinhibits DA neurons and promotes DA release in the NAc. Chronic exposure to GHB **(b)** reduces RGS2 expression in DA neurons, strengthening the coupling efficiency of $GABA_B$–GIRK. In this situation, acute administration of GHB also stimulates $GABA_B$ receptors coupled to GIRK channels in DA neurons, thereby directly inhibiting DA activity and reducing DA release in the NAc. The unique expression of GIRK2 and GIRK3 channels in DA neurons, the lower $G\beta\gamma$ affinity of GIRK2/3 heteromeric channels and the selective association of RGS2 with GIRK3 enable the up or downregulation of DA activity.

substantially enhances the coupling efficiency between $GABA_B$ receptors and GIRK channels in the DA neurons of the VTA. This shift in the EC_{50} to lower concentrations (increase in coupling efficiency) is sufficient such that low concentrations of GHB, which normally do not activate GIRK currents in DA neurons, can now hyperpolarize DA neurons and decrease DA firing rates. Remarkably, this decrease in DA-neuron excitability is associated with a behavioral loss of drinking preference for GHB.

The mechanism by which addictive drugs change RGS expression is not well understood, but is likely to involve DA receptors (D_1 and D_2), activation of cAMP via adenylyl cyclase, protein-kinase-A-dependent phosphorylation and cAMP-response-element-binding protein [34–37]. RGS regulation via DA receptors might be unique for RGS2, which is the only member of the RGS family found, thus far, to contain a cAMP-responsive promoter region. During withdrawal, when cAMP levels are elevated because of super-sensitization of adenylyl cyclase, RGS2 levels could quickly recover and exceed baseline values owing to the cAMP-responsive promoter region. More studies are needed to delineate the mechanism of drug-dependent changes in RGS expression.

Conclusions

Numerous studies have shown that sustained exposure to addictive drugs can regulate mRNA expression of several members of the RGS family. We have recently discovered that drug-dependent downregulation of RGS2 protein substantially enhances coupling efficiency of $GABA_B$ receptors with GIRK channels. These changes were associated with a polarity switch in the output of the VTA, in which the behavioral response to GHB is converted from reinforcing to aversive in animals chronically treated with GHB. The unique expression of GIRK2/3 channels and RGS2 proteins in the VTA–DA neurons, combined with selective molecular interactions between GIRK3 and RGS2 in a signaling complex, enable the electrophysiological and behavioral polarity switch in DA neurons from the VTA. These studies highlight RGS proteins as powerful regulators of GPCR–GIRK coupling efficiency and indicate a mechanism for a special form of tolerance (Box 1).

Demonstrating that RGS proteins participate in the adaptive response to addictive drugs indicates RGS proteins could be novel targets for treating addiction. Harnessing the change in coupling efficiency in DA neurons could provide a new avenue of research for treating addiction. For example, selective inhibition of the GAP domain in RGS2 would be expected to enhance GPCR signaling in the VTA, reducing DA-neuron excitability. Alternatively, selective inhibition of RGS proteins could modify tolerance and dependence to certain drugs. More selective pharmacological tools will be needed, however, to avoid negative side effects. Future studies developing inhibitors targeted to some of the unique domains in RGS proteins could be one solution [38]. Lastly, if RGS-dependent changes in coupling efficiency between GPCRs and other effectors are found, then RGS proteins might become interesting targets for a wide range of pathologies, from drug addiction to hypertension and heart disease.

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