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A role of RGS proteins in drug addiction

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ABSTRACT

The diverse family of Regulators of G protein signaling (RGS) proteins are widely distributed proteins with multiple functions, including GAP activity for heterotrimeric G protein alpha subunits. Three members of the RGS family, RGS9-2, RGS4 and RGSz, have been shown to play an essential modulatory role in psychostimulant and opiate drug actions. Interestingly, these proteins show distinct structure, distribution pattern and cellular localization. In addition, each of these proteins is differentially regulated by drugs of abuse in particular brain networks and appears to modulate distinct signal transduction events. The striatal enriched RGS9 plays a prominent role in opiate and psychostimulant drug reward; RGS4 appears to modulate opiate dependence via actions in the locus coeruleus, whereas RGSz modulates analgesia via activation of the PKC pathway.

Drug addiction is a chronic disease, triggered by repeated exposure to substances such as opioids and psychostimulants, and it is generally defined as compulsive drug use and loss of control over drug intake [1–5]. Exposure to drugs of abuse affects the function of several brain networks including the mesoaccumbens dopamine pathway, extending from the ventral tegmental area of the midbrain to the nucleus accumbens (ventral striatum) and from there to the prefrontal cortex [6]. The addiction process also involves several nuclei of the amygdala [2,7], the hippocampus [8,9] the locus coeruleus [2,10] the periaqueductal gray [8] and the spinal cord [11,12]. The neurochemical changes that follow exposure to addictive substances involve the dopaminergic, opioidergic and noradrenergic systems. Each of these neurotransmitter systems function by binding and activating cell surface G protein-coupled receptors (GPCRs) expressed in pre-synaptic or post-synaptic cell membranes, resulting in the initiation of cellular signaling events that mediate neuronal responses to these neurotransmitters. GPCRs are a major class of transmembrane receptors that are targeted in greater than half of all pharmaceutics on the market and nearly all drugs of abuse. Many of the adaptive changes that occur during the progression of addiction involve GPCRs and other molecules that regulate the activity of G proteins or their downstream effectors.

GPCRs and the cellular proteins with which they are associated transmit signals across the plasma membrane through conformational changes, enzymatic activity, and formation of multi-protein complexes. The immediate effect of receptor activation by ligand is the activation of heterotrimeric G proteins, which act as molecular switches in diverse cell signaling pathways [13]. When bound to guanine nucleotide diphosphate (GDP), the Gα subunit associates with a Gβγ dimer in an inactive heterotrimer. When bound to guanine nucleotide triphosphate (GTP) the Gα subunit undergoes conformational changes in three switch regions involved in binding to the Gβγ dimer and to effector molecules. The Gα subunit then dissociates from the Gβγ dimer, both of which

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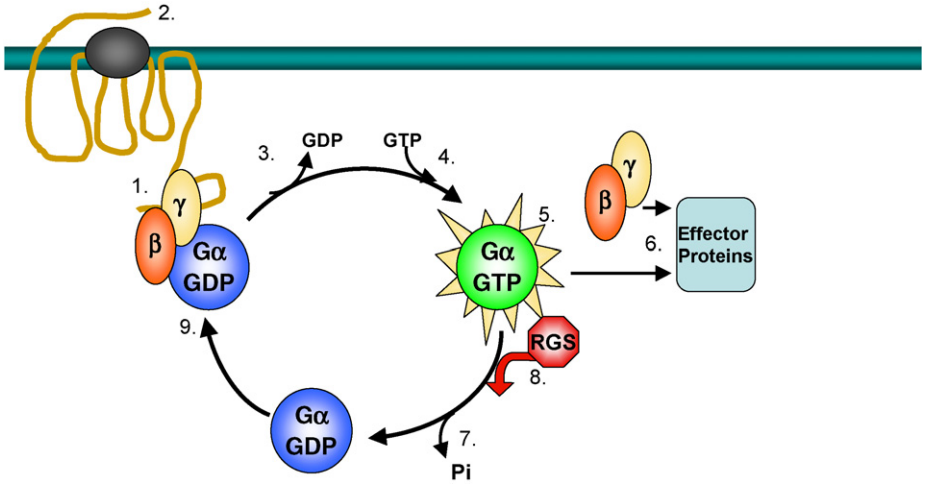

Fig. 1 – G-protein nucleotide binding and activation cycle. In the basal state, the Gα subunit is bound to GDP and forms an inactive heterotrimer with Gβγ dimers. The heterotrimer associates with cytosolic portions of GPCRs to promote the high-affinity state of the receptor (1). Agonist binds receptor, inducing conformational changes in the transmembrane domains of the receptor (2). These conformational changes alter receptor interaction of the G-protein heterotrimer in such a way to promote the dissociation of GDP from the Gα nucleotide binding pocket (3). Once GDP is removed from the binding pocket, GTP, which is present in cytosol at much higher concentrations than GDP, quickly binds the Gα subunit, causing major changes in the conformation of three helical "switch regions" of the Gα subunit (4). The switch regions of Gα contain critical contact points for association with Gβγ dimers. Structural changes in these regions induced by GTP binding cause dissociation of Gβγ dimers from the Gα subunit (5). Dissociation of the heterotrimer unmasks active binding sites on both the Gα subunit and the Gβγ dimer that are responsible for activating effector enzymes that regulate neuronal activity (6). The Gα subunit functions as a GTPase enzyme, hydrolyzing the third phosphate group of GTP to generate GDP in the nucleotide binding pocket (7). While the endogenous Gα GTPase activity is very slow, this rate is accelerated dramatically by RGS proteins, which directly bind GTP-bound Gα subunits and enhance their GTPase activity (8). Once returned to the inactive GDP bound state, Gα subunits reassociate with Gβγ dimers to return to the basal state (9).

are then active to trigger downstream signaling events. Receptors activate specific subtypes of G proteins, each of which mediates neuronal responses to the neurotransmitter signal by regulating second messenger levels or by directly regulating ion channel function. There are four major classes of Gα G protein subunits, which differ in their effects on downstream effectors. Briefly, $G\alpha_s$ proteins activate adenylyl cyclase to increase cellular cAMP concentrations; $G\alpha_{i/o}$ proteins inhibit adenylyl cyclase to oppose $G\alpha_s$ activity, activate G protein regulated inwardly rectifying potassium (GIRK) channels, and inhibit voltage-gated calcium channels; $G\alpha_q$ proteins activate phospholipase C enzymes to produce diacyl glycerol and inositol triphosphate, and inhibit GIRK channels; $G\alpha_{12/13}$ proteins activate the small G protein Rho and some phospholipase subtypes; and Gβγ dimers activate phospholipases and ion channels (reviewed in [10,14–16]). Thus, there is remarkable functional diversity among G protein families [17].

Both the activation and deactivation of G proteins are highly regulated events in cells. Neurotransmitter binding to the receptor initiates conformational changes in the transmembrane domains, particularly TM3 and TM6 [18], that interact with heterotrimeric G proteins. Upon ligand activation, GPCRs stimulate exchange of GDP for GTP, thereby turning G proteins "on". The lifetime of this active state of G proteins is regulated in cells by the rate at which the Gα subunit hydrolyzes GTP, and thereby returns to the GDP bound inactive state. G protein alpha subunits possess weak GTPase catalytic activity, but the basal rates of this activity are much slower than the deactivation kinetics of G proteins in vivo. Accounting for this discrepancy, diverse GTPase accelerating proteins (GAPs) function to enhance the GTPase activity of the G-α subunit [19], thereby turning G proteins "off" (Fig. 1). While GPCRs and their ligands have been extensively investigated as G protein activators, relatively little is known about the more recently discovered proteins that deactivate G proteins. A diverse family of Regulator of G protein signaling (RGS) proteins that act as GAPs for heterotrimeric G protein α subunits has emerged over the last decade [20–22]. By accelerating the return of the activated GTP-bound Gα protein to its basal GDP-bound state, RGS proteins terminate effector activation by both Gα and Gβγ subunits, thus regulating the kinetics and amplitude of signaling.

1. GPCR families in addiction biology

Changes in dopamine receptor signaling in the nucleus accumbens underlie addiction to many abused drugs. To date, more than five dopamine receptor subtypes have been found to be expressed in the mesolimbic dopamine system [23]. Dopamine receptor subtypes are linked to different G proteins, with D_1 and D_5 associated with G_s activity, whereas D_2-like receptors (D_2, D_3, D_4) are thought to act primarily via