Presynaptic Signaling by Heterotrimeric G-Proteins

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Abstract G-proteins (guanine nucleotide-binding proteins) are membrane-attached proteins composed of three subunits, α, β, and γ. They transduce signals from G-protein coupled receptors (GPCRs) to target effector proteins. The agonist-activated receptor induces a conformational change in the G-protein trimer so that the α-subunit binds GTP in exchange for GDP and α-GTP, and βγ-subunits separate to interact with the target effector. Effector-interaction is terminated by the α-subunit GTPase activity, whereby bound GTP is hydrolyzed to GDP. This is accelerated in situ by RGS proteins, acting as GTPase-activating proteins (GAPs). Gα-GDP and Gβγ then reassociate to form the Gαβγ trimer. G-proteins primarily involved in the modulation of neurotransmitter release are G\textsubscript{o}, G\textsubscript{q} and G\textsubscript{s}. G\textsubscript{o} mediates the widespread presynaptic auto-inhibitory effect of many neurotransmitters (e.g., via M2/M4 muscarinic receptors, α\textsubscript{2} adrenoreceptors, μ/δ opioid receptors, GABA\textsubscript{B} receptors). The G\textsubscript{o} βγ-subunit acts in two ways: first, and most ubiquitously, by direct binding to Ca\textsubscript{v2}Ca\textsuperscript{2+} channels, resulting in a reduced...
sensitivity to membrane depolarization and reduced Ca\(^{2+}\) influx during the terminal action potential; and second, through a direct inhibitory effect on the transmitter release machinery, by binding to proteins of the SNARE complex. \(G_s\) and \(G_q\) are mainly responsible for receptor-mediated facilitatory effects, through activation of target enzymes (adenylate cyclase, AC and phospholipase-C, PLC respectively) by the GTP-bound \(\alpha\)-subunits. AC generates cyclic AMP which activates protein kinase A (PKA) and PLC hydrolyzes membrane phosphatidylinositol-4,5-bisphosphate (\(\text{PIP}_2\)) to form diacylglycerol (DAG) to activate protein kinase C (PKC). PKC phosphorylates Ca\(^{2+}\) channel proteins to oppose \(G_0\beta\gamma\)-mediated inhibition, and both PKA and PKC phosphorylate various components of the release machinery to enhance exocytosis. cAMP and DAG can themselves facilitate release by direct, phosphorylation-independent, regulation through second messenger binding proteins, including cAMP-GEFs and Munc-13 (DAG-binding). Finally, membrane levels of \(\text{PIP}_2\) play a signaling role throughout the stimulus-secretion cascade.

1 Overview of G Protein Signaling

Heterotrimeric G-proteins are guanine nucleotide-binding membrane-associated proteins that directly intermediate between the G-protein-coupled (heptahelical) receptor and the target effector protein. They are composed of \(\alpha\), \(\beta\) and \(\gamma\) subunits. The trimer is anchored in the membrane via palmitoyl or myristoyl fatty acids at the N-terminus of the \(\alpha\) subunit and a prenyl moiety at the C-terminus of the \(\gamma\) subunit (see Gilman 1987; Oldham and Hamm 2006 for details).

The basic cycle of G-protein activation and inactivation is illustrated in Figure 1. In the basal state (1) the \(\alpha\)-subunit of the trimer binds guanosine diphosphate (GDP). On activation by agonist (Ag) the receptor “docks on” to the G-protein (2) and induces a conformational change in the latter that exposes a high-affinity guanosine triphosphate (GTP) binding site on the \(\alpha\)-subunit. Since the concentration of GTP in the cytosol exceeds that of GDP, this promotes a GTP-GDP nucleotide exchange (the rate-limiting process for activation). This conformational change also induces a partial (see below) or complete dissociation of the \(\alpha\) and \(\beta\gamma\) subunits, though both usually stay anchored in the membrane (except the \(\alpha\)-subunit of rod transducin) (Calvert et al. 2006; Rosenzweig et al. 2007). The \(\alpha\) and \(\beta\gamma\) subunits then separately (or sometimes conjointly) interact with one or more effector proteins, to activate or inhibit them (3). The cycle is completed by the hydrolysis of \(\alpha\)-attached GTP to GDP by the GTPase activity of the \(\alpha\)-subunit; the GDP-bound \(\alpha\)-subunit then reassociates with the \(\beta\gamma\)-subunit to restore the ground state. Clear dissociation of \(\alpha\) and \(\beta\gamma\) subunits occurs in the visual transduction pathway, and has also been shown for the \(G_o\) trimer (Tesmer et al. 2005); however some evidence (Bunemann et al. 2003; Gales et al. 2006; Levitzki and Klein 2002; Rosenzweig et al. 2007) suggests that the \(\alpha\) and \(\beta\gamma\) subunits do not necessarily dissociate in other systems but stay