

Appendix A14.13

Cellular Signal Transduction Systems: A Primer

Signal Transduction: What is It?

An essential property of any living cell is its ability to recognize and respond to external stimuli. Cell surface receptors have two major functions: recognition of specific molecules (neurotransmitters, hormones, growth factors and even sensory signals) and activation of “effectors”. Binding of the appropriate neurotransmitter or hormone externally to the receptor alters the molecular configuration of the protein. Cell surface receptors use a variety of membrane transducing mechanisms to transform an agonist's message into cellular responses. In neuronal systems, the most typical responses involve changes in transmembrane voltage and hence neuronal changes in excitability. Collectively, the processes are referred to as transmembrane signaling or signal transduction mechanisms.

Interestingly, although increasing numbers of potential neurotransmitters and receptors continue to be identified, it has become clear that the translation of the extracellular signals (into a form that can be interpreted by the complex intracellular enzymatic machinery) is achieved through a relatively small number of molecular mechanisms. Generally speaking, these transmembrane signaling systems, and the receptors that utilize them, can be divided into three major groups:

- a) those which are relatively self-contained in structure and whose message takes the form of transmembrane ion fluxes;
- b) those which are multicomponent in nature and generate intracellular second messengers
- c) those that contain intrinsic enzymatic activity (e.g. receptor tyrosine kinases and phosphatases) (these will be discussed in the appendix dealing with neurotrophic signaling cascades).

The first class of receptors contains in their stable molecular complex an intrinsic ion channel. Receptors of this class include those for a number of amino acids including glutamate, GABA (gamma aminobutyric acid via the GABA_A receptor) glycine, the nicotinic acetylcholine receptor, the NMDA receptor, and the 5HT₃ receptor. Ion channels are integral membrane proteins directly responsible for the electrical activity of the nervous system by virtue of their regulation of the movement of ions across membranes. Receptors containing intrinsic ion channels have been called “ionotropic” and are generally composed of four or five subunits which

open transiently when neurotransmitter binds, allowing ions to flow into (e.g. Na^+ , Ca^{2+} , Cl) or out of (e.g. K^+) the neuron, thereby generating synaptic potential. Neurotransmission of this type is very fast, with ion channels opening and closing within milliseconds, and is classically employed by those neurotransmitters mediating sensation or controlling movement. A variety of sedative-hypnotic agents (including benzodiazepine, barbiturates and ethanol) appear to exert their major effects at such ionotropic receptor complexes. Indeed, perhaps the single most important factor contributing to the rapid advances in understanding the mechanism(s) of action of anxiolytics is the progress made recently in unraveling the molecular mechanism(s) of action of the major class of anxiolytic drugs, the benzodiazepines.

Benzodiazepines are known to produce their actions by first interacting with a specific neuronal protein (the benzodiazepine receptor) that is functionally associated with a recognition site (receptor) for the major inhibitory neurotransmitter in brain, gamma-aminobutyric acid (GABA). It has been estimated that this neurotransmitter is involved in 40% of all synapses, making it the most ubiquitous of all neurotransmitters. Any drugs potentiating GABA have widespread inhibitory actions. Benzodiazepines do not act directly on GABA receptors but, as noted above, have their own receptors which form (in association with other regulatory proteins) the so-called "GABA/benzodiazepine/chloride ionophore". Benzodiazepines, barbiturates, and ethanol all appear to regulate the transport or permeability of chloride across neuronal membranes, thereby making the neuron less excitable (hyperpolarizing) and inhibiting firing.

Metabotropic Receptors

Receptors at which many major classes of drugs--including antipsychotics and antidepressants--act do not, however, have ionic conductance channels within their structure but instead regulate cellular activity by the generation of various "second messengers." In general, receptors of this class do not directly interact with the various second messenger generating enzymes but transmit information to the appropriate "effector" by the activation of interposed "coupling proteins". It is now known that receptors for a large number of circulating hormones and neurotransmitters transduce the signal of agonist binding at the extracellular side of the membrane to intracellular effectors via intermediary proteins (referred to as G proteins). Receptors coupled to guanine nucleotide binding (hence G) proteins include those for catecholamines, serotonin, acetylcholine, various peptides, and even sensory signals such as light and odorants. G protein coupled receptors are often referred to as "metabotropic" receptors. Activation of these receptors initiates a cascade of events via regulation of intracellular second messengers (e.g. cAMP), usually leading to phosphorylation of various membrane and cytosolic proteins. As might be predicted, G protein coupled receptors generate electrical signals on a much slower time scale, often with a latency of onset of signal of at least 30 milliseconds. The ability of G proteins to interact with multiple receptors also provides an elegant mechanism for

the neurons' integrative functions; this has led to the proposal that G protein coupled receptors may be involved in pathways regulating such diverse complex pathways regulating human behavior.

G Protein Networks Function as Signal Amplifiers and Integrators

With the exception of synaptic transmission mediated via receptors that contain intrinsic enzymatic activity or that form ion channels, the family membrane proteins known as G proteins appears to be involved in all other transmembrane signaling in the nervous system. G proteins, first identified and characterized by Rodbell, Gilman, and others, are named because of their ability to bind the guanine nucleotides, guanosine triphosphate (GTP) and guanosine diphosphate (GDP). The proteins also possess an intrinsic GTPase activity (ie the ability to cleave the third phosphate group off GTP, thereby producing GDP in a “turn-off” reaction). Many types of effector proteins are known to be influenced by G proteins; these include ion channels, adenylyl cyclase, phospholipase C (which catalyzes the hydrolysis of phosphatidylinositol), phospholipase A₂ (which catalyzes the hydrolysis of arachidonic acid), and phosphodiesterase (PDE) (in rod outer segments).

G Proteins are a Family of Heterotrimeric Proteins

Three types of G protein were identified in early studies G_t, termed “transducin,” was identified as the G protein that couples rhodopsin to regulation of photoreceptor cell function, and G_s and G_i were identified as the G proteins that couple plasma membrane receptors to the stimulation and inhibition, respectively, of adenylyl cyclase, the enzyme that catalyzes the synthesis of cAMP. Since that time, a multitude of G protein subunits have been identified by a combination of biochemical and molecular cloning techniques. In addition to G_t, G_s, and G_i, the other major types of G protein in brain are designated G_o, G_{olf}, G_{gust}, G_z, G_q, and G₁₁₋₁₆. The subunits are named according to decreasing mass, with the α -subunits having an apparent mass of 40-52 kDa, the apparent mass of β -subunits is 35 to 36 kDa; that for the γ -subunits is 5 to 20 kDa. The α subunits had previously been assumed to confer receptor and effector specificity to the G protein (and indeed, the G proteins are named according to their α subunits), but recent evidence has also demonstrated critical roles for $\beta\gamma$ subunits in signal transduction pathways.

Information traverses catalytically across a G protein coupled system in a GTPase cycle, resulting in a several thousand fold amplification of the original signal. The G protein α subunits have intrinsic GTPase activity that cleaves bound GTP to GDP, and interactions with certain effectors, and a novel class of proteins (RGS—regulators of G protein signaling) accelerates intrinsic α subunit GTPase activity. GTP hydrolysis serves to “turn off” the G protein and allows

reassociation of the subunits of the heterotrimer . Indirectly, this also contributes to the desensitization process.

G Proteins Regulate Major Second Messenger Regulated Protein Kinase Cascades

The cAMP Second Messenger Generating System

The G protein-linked signal transduction pathway involving the enzyme adenylyl cyclase (also referred to as adenylyl cyclase) is well characterized. Adenylyl cyclase, of which there are several distinct forms, is an enzyme that converts ATP to the second messenger, cyclic cAMP. Depending on the particular G protein coupled receptor and linked G protein, cAMP is either up or downregulated. As mentioned previously, G_s is involved in stimulating adenylyl cyclase whereas G_i inhibits this enzyme. Most receptors that regulate cAMP action do so via their effect on one of these G proteins.

A recognized effect of cAMP is activation of protein kinase A (PKA), an enzyme that phosphorylates and regulates many proteins including ion channels, cytoskeletal elements, transcription factors, and other enzymes. One of the transcription factors phosphorylated and thereby modulated by PKA is the cAMP response element binding protein (CREB). This protein binds to the cAMP response element (CRE), a gene sequence found in the promoter of certain genes. CREB also regulates a number of processes in neurons including neuronal excitation, development, apoptosis, and long-term synaptic plasticity. Variability of the response of CRE to CREB binding in individual cells is regulated by additional transcription factors that are under the control of cAMP and other cellular signals. Cellular processes downstream of PKA include phosphodiesterases—proteins that change the activity of cAMP by converting it to AMP—and numerous phosphatases that may remove phosphate groups from the proteins phosphorylated by PKA.

The Phosphoinositide/Protein Kinase C Signal Transduction Pathway

Although inositol phospholipids are relatively minor components of cell membranes, they play a major role receptor-mediated signal-transduction pathways, involved in a diverse range of responses such as cell division, secretion, and neuronal excitability and responsiveness. In brief, agonist-stimulated phosphatidylinositol-4,5-bisphosphate (PIP₂) hydrolysis has been extensively reviewed elsewhere, and the interested readers are referred to these for details (Baraban et al., 1989; Berridge et al., 1989; Catt and Balla, 1989; Chuang, 1989; Irvine, 1989; Rana and Hokin, 1990; Fisher et al., 1992). Agonists such as acetylcholine, norepinephrine, serotonin, bradykinin or vasopressin bind to specific cell surface receptors, which interact with both pertussis toxin sensitive (G_o and/or G_i) and pertussis toxin insensitive (G_q/G_{11}) guanine

nucleotide binding proteins (G proteins), and thereby stimulate the enzyme phospholipase C (PLC), to produce the intracellular second messengers sn-1,2-DAG (the endogenous activator of PKC) and inositol-1,4,5-trisphosphate (IP3). IP3 stimulates the release of intracellular stored Ca^{++} from the smooth endoplasmic reticulum, perhaps from a specialized store termed the calciosome. Ca^{++} release is stimulated by the binding of IP3 to a receptor specific for the 1,4,5 isomer. IP3 can be metabolized both by dephosphorylation to form Ins 1,4P2, or phosphorylated to form Ins 1,3,4,5P4 (IP4), which has been proposed to be involved in the entry of Ca^{++} into cells from extracellular sources. Crucial to this paper is the fact that the ability of a cell to maintain sufficient supplies of myo-inositol is crucial to the resynthesis of the phosphoinositides and the maintenance and efficiency of signaling. In several cell types, and especially in the CNS, the conservation of inositol depends strongly upon recycling via dephosphorylation of inositol phosphates. Since the metabolite of phosphatidic acid, CMP-PA combines with free myo-inositol to resynthesize inositol phosphates for the PI cycle, depletion of cellular inositol interferes with the recycling of the system, resulting in the consequent accumulation of CMP-PA and its interconvertible metabolite (and endogenous activator of PKC), diacylglycerol

Protein kinase C

Ca^{++} activated, phospholipid-dependant protein kinase [protein kinase C (PKC)] is a ubiquitous enzyme, highly enriched in brain, where it plays a major role in regulating both pre- and postsynaptic aspects of neurotransmission (Nishizuka, 1988, 1992; Stabel and Parker, 1991; Newton, 1995). PKC is one of the major intracellular mediators of signals generated upon external stimulation of cells via a variety of neurotransmitter receptors (including muscarinic m1 and m3, noradrenergic $\alpha 1$, serotonergic 5HT2c) which induce the hydrolysis of various membrane phospholipids. Activation of PKC by DAG appears to involve the binding of the lipid to a specific regulatory site on the enzyme, resulting in an increase in the Ca^{++} affinity of the enzyme, and thus its stimulation at physiological ionic concentrations. Ca^{++} is also believed to contribute to PKC activation by facilitating the interaction of the enzyme with the lipid bilayer and hence with acidic phospholipids and DAG. This appears to be the primary mechanism for initiating PKC-mediated events, but increasing recent evidence has indicated that signal-dependent hydrolysis of additional phospholipids (including the most abundant membrane phospholipid, phosphatidylcholine) may also provide DAG, in the absence of PI turnover. Indeed, recent studies have indicated that many of the agonists that stimulate PIP2 hydrolysis also promote phosphatidylcholine breakdown, through the activation of phospholipases of the C and D type to yield DAG, phosphatidic acid, choline, and phosphorylcholine (Exton, 1990; Billah & Anthes, 1990).

PKC is now known to exist as a family of closely related subspecies, has a heterogeneous distribution in brain (with particularly high levels in presynaptic nerve terminals), and together

with other kinases, appears to play a crucial role in the regulation of synaptic plasticity and various forms of learning and memory. At present, PKC is the largest family of serine/threonine specific kinase subfamily known, rivaling in size the expansive src related tyrosine specific protein kinase family. The comparison between src and PKC families is not trivial and bears upon why nature has gone to so much trouble during evolution to diversify and conserve these families of regulatory proteins. As transducers involved in signal transduction processes, such diversification can, in theory, serve to modify the nature of the input, the output or the flux through the transducer. The multiple, closely related PKC isoforms are all activated by phospholipids and DAG, albeit with slightly different kinetics. The isoforms can be subclassified according to Ca⁺⁺ dependence; the “conventional” PKCs (α , β I, β II, γ) are dependent on Ca⁺⁺ for activity, whereas at least four others (δ , ϵ , η , ζ -sometimes called the “novel” PKC family) are calcium independent (Nishizuka, 1988). The differential tissue distribution of PKC isozymes as well as the fact that several isoforms are expressed within a single cell type, suggests that each isozyme may exert distinct cellular functions. At present, it is unclear whether such putative functional specificity arises from differential *in vivo* activation, differential substrate specificity, or a combination thereof.

G Proteins Can Directly Couple to Selected Ion Channels in the CNS

In many cases, it appears that the α - or $\beta\gamma$ subunits released from the G protein-receptor interaction directly gates (i.e., opens or closes) a specific ion channel. One of the best-established examples of this type of mechanism in brain is the coupling of opiate, α_2 -adrenergic, D₂ dopaminergic, muscarinic cholinergic, 5-HT_{1A} serotonergic, and GABA_B receptors to the activation of an inward rectifying K⁺ channel via pertussis toxin-sensitive G proteins (i.e., subtypes of G_o and/or G_i). Some of these same neurotransmitter receptors have been shown to be similarly coupled to voltage-dependent Ca²⁺ channels via the same types of G proteins, although in this case a direct action of $\beta\gamma$ complexes on ion channels has been proposed.

Regulation of G Proteins by Hormones

The CNS is a major target for the actions of glucocorticoids, thyroid hormones, and gonadal hormones, but the biochemical alterations ultimately responsible for producing the effects remain unclear. Malbon and associates coined the term “permissive hormones” to describe agents such as glucocorticoids and thyroid hormones which modulate the actions of a variety of agents acting through cAMP. Increasing evidence suggests that these modulatory actions are exerted to a significant degree via G protein regulation. Thus, *in vivo* alterations of hormonal levels have been demonstrated to alter the steady state levels of several G protein subunits and thereby regulate the overall sensitivity of transmembrane signaling in a variety of peripheral tissues. Less information is available about the effects of hormonal manipulation on G

protein regulation in the CNS. However, it has been demonstrated that 7-day administration of corticosterone increases the transcription and expression of G_{α} , while decreasing both the mRNA and immunoreactivity of $G_{i\alpha}$ in rat cerebral cortex. Moreover, adrenalectomy without corticosterone replacement results in a significant 20% decrease in G_{α} mRNA, suggesting in vivo physiologic regulation. More recently, it has been demonstrated that pretreatment of mouse striatal neurons in primary culture with 17 β -estradiol or testosterone increased the pertussis toxin-catalyzed ADP-ribosylation of $G_{\alpha o,i}$ subunits.

Abundant evidence supports an interaction between thyroid and adrenal hormones and psychiatric illness, particularly in affective disorders. Thus, primary disorders of both the thyroid and HPA axis have been linked with depressive, manic and anxiety symptoms. Additionally, there is general agreement that more than 50% of patients with major depression exhibit hyperactivity of the HPA axis with hypercortisolemia, ACTH hypersecretion and non-suppression of plasma cortisol by dexamethasone. Similarly, at least some studies report the administration of thyroid hormones to be of benefit in the treatment of refractory depression and rapid cycling bipolar disorder. Although a variety of biochemical effects may contribute to the CNS manifestations of alterations in thyroid or corticosteroid status, alterations of G protein function or content (with the inherent amplification on receptor responses) by these “permissive hormones” represents an attractive mechanism. Similarly, the effects of gonadal hormones on G proteins, resulting in subsequent modification of signal transduction, may be one mechanism by which biological maturation (puberty or menopause) triggers the expression of certain psychotic illnesses (e.g., schizophrenia in late adolescence, melancholic depression in late adulthood).

G Proteins as Mediators of Neurotransmitter-Neurotransmitter and Receptor-Receptor Interactions

Most clinical studies in psychiatry, even when multiple measures are obtained, analyze data primarily in terms of independence of measures; i.e., is norepinephrine deficient? Is serotonin deficient, and so forth. However, considerable preclinical evidence shows that monoamine systems interact, and a major question for neuroscience is emerging with regard to elucidating the mechanism(s) by which one neurotransmitter influences the response of a neuron to all the other converging afferent inputs. The CNS is remarkably complex, both anatomically and chemically, with a remarkable convergence of different receptors in common cortical layers and a considerable convergence of neurotransmitter action. A single neuron in the brain receives thousands of synaptic inputs on the cell body and dendrites, and neuronal response is also modulated by a variety of hormonal and neurohormonal substances not dependent on synaptic organization. The neuron needs to integrate all the synaptic and non-synaptic inputs impinging upon it; this integration of a multitude of signals determines the ultimate excitability, firing pattern and response characteristics of the neuron, which is then conveyed to succeeding targets

via synaptic transmission. How does the single neuron decipher and integrate the multitude of signals it receives and, additionally, generate unique responses to each of these signals or combinations of signals? Not only do G proteins amplify signals, but they also appear to form the basis of a complex information processing network in the plasma membrane. Thus, the ability of G proteins to interact with multiple receptors provides an elegant mechanism to organize the signals from these multiple receptors and to transmit them to a relatively much smaller number of effectors. Signals from a variety of receptors can be “weighted” according to their intrinsic ability to activate a given G protein and integrated to stimulate a single second messenger pathway. Similarly, the dual (positive and negative) regulation of adenylyl cyclase and perhaps of phospholipase C by G proteins allows for stimulatory and inhibitory signals for these pathways to be “balanced” at the G protein level, yielding an integrated, output. Convergence of a variety of neurotransmitters on the same ion channel has been demonstrated to occur in the locus coeruleus, hippocampus, thalamus, and substantia nigra. Thus, it appears that a variety of receptors that coexist on neurons and mediate similar responses may share signal transduction mechanisms. This convergence may occur at the level of the G protein or the effector itself. In both the cortex and the hippocampus, α_2 adrenergic, adenosine A₁, and K-opiate receptors appear to compete for the same pool of G proteins to modulate Ca⁺² influx via voltage sensitive Ca⁺² channels, and thereby decrease NE release. Additionally, there is a mutually antagonistic effect between these receptors with respect to modulating both Ca⁺² influx and NE release. That is, stimulating one of these receptors diminishes the effectiveness of the other receptors; this may represent a mechanism that allows neurons to “escape” from excessive inhibitory input. Similarly, heterologous desensitization (a form of desensitization whereby exposure to a desensitizing agent leads to a diminished responsiveness to a number of stimuli and appears to involve post-receptor mechanisms) may be a compensatory mechanism designed to protect the neuron from the deleterious effects of overstimulation by multiple receptors. Thus, G proteins provide the first opportunity for signals from different receptors to be integrated; this complex web of interactions linking receptors, G proteins, and their effectors with signals converging to shared detectors appears to be crucial for the integrative functions performed by the CNS.

G Proteins as Mediators of Receptor-Effector Cross-Talk in the CNS

In addition to their role as receptor-effector couplers, G proteins also serve as targets for regulating cross-talk between various second messenger systems. There are a number of potential sites of PKC interaction with the cAMP generating system. A large body of evidence suggests that phosphorylation of the β AR by various kinases, including PKC, desensitizes the receptor. Increasing evidence has also accumulated that the potentiating effects of PKC on cAMP accumulation are mediated by Gi. Thus, in a number of cell types, including hepatocytes and

striatal membranes, PKC activation appears to enhance AC activity by attenuating the inhibitory influence of Gi. Since the susceptibility of G proteins to be phosphorylated (and therefore regulated) by PKC depends in large part on their conformational state, the degree of cross-talk is regulated by the relative degree of simultaneous stimulation of various receptors. In sum, it is clear that interactions between distinct second messenger generating systems represent a “fine-tuning” cellular network which regulates the neuron's reactions to the large number of extracellular signals that it encounters. The “net effect” of the various potential interactions probably depends on the summation of the effects on individual components. In this context, quantitative and qualitative (e.g. conformation states) differences among subtypes of G proteins and relative abundance of PKC isozymes in various cells may be major parameters in determining the final integrated output.

Clinical Disorders Arising from Abnormalities in G Protein Coupled Signaling Pathways

There is a growing appreciation that the “molecular medicine revolution” has resulted in a more complete understanding about the etiology and pathophysiology of a variety of medical disorders, is largely attributable to the elucidation of the basic mechanisms of signal transduction, and that the application of the powerful tools of molecular and cellular biology to the study of human disease (Weintraub, 1995; Spiegel, 1998). This has allowed the study of a variety of human diseases which are caused by loss- and gain-of-function mutations; studies of such diseases are offering unique insights into the physiologic and pathophysiologic functioning of many cellular signaling pathways and networks. Indeed, given their widespread and crucial role in the integration, regulation, and amplification of signal transduction pathways, investigators were not sure if abnormalities in these critical regulators of organismic function would even be compatible with life.

However, alterations in the function and/or expression of various G proteins have been recently implicated in a variety of pathophysiological states. What is particularly interesting is that is the observation that a variety of diseases manifest relatively circumscribed symptomatology, despite the widespread expression of the affected signaling proteins. This raises the distinct possibility that disorders with primarily CNS manifestations can arise from genetic or acquired abnormalities in critical signal transduction pathways which are ubiquitously expressed. Thus, as we discuss below, there is considerable precedence for clinical disorders arising from abnormalities in the levels of G α s, which present with limited clinical manifestations, despite the ubiquitous expression of G α s (discussed in Spiegel et al., 1992; Manji et al., 1995b). These heterogeneous effects have been postulated to arise from differences in receptor, G protein, and effector stoichiometries in different tissues and to differences in the ability of different cells to compensate for the abnormality.

The first disease in which an intrinsic G protein abnormality was found was pseudohypoparathyroidism (called “pseudohypoparathyroidism“ because the individuals have normal [or even increased] levels of parathyroid hormone, but have the manifestations of the disease because of cellular resistance to the hormone). In one form of the illness (termed Albright's Hereditary Osteodystrophy), the patients demonstrate a resistance to parathyroid hormone and to a number of hormones utilizing cAMP as the second messenger. In these patients, reduced expression or function (approximately 50%) of $Gs\alpha$ has been demonstrated in cultured cells as well as from freshly obtained tissue. Additionally, distinct mutations have been identified in the gene encoding $Gs\alpha$ in different kindreds with the disease, thereby providing the first demonstrations of an inherited mutation in a human G protein gene with important implications. The existence of more than one type of mutation resulting in altered function of $Gs\alpha$ demonstrates the genetic heterogeneity present in this autosomal dominant disease. Moreover, the presence of an identical mutation in the $Gs\alpha$ gene, both in individuals with multiple hormone resistance and in those without hormone resistance, clearly suggests that Gs deficiency is necessary but not sufficient for the full phenotypic expression of the disease. Thus (as has been suggested for a variety of psychiatric disorders) additional factors, including “modifying genes” and perhaps environmental factors, appear to be involved.

Recent studies have also demonstrated significant alterations in G proteins in failing human and animal heart. Thus, a 50% decrease in the apparent concentration and function of Gs in sarcolemma from a canine model of left ventricular failure has recently been reported. In contrast, end-stage idiopathic human congestive failure is associated with increased activity of G_i , suggesting that various manipulations of Gs/ G_i stoichiometry may result in similar pathophysiology . Perhaps more akin to psychiatric research with respect to the use of peripheral blood cells to represent less accessible tissue (CNS, heart), one study reported an 80% decrease in lymphocyte Gs levels in subjects with congestive heart failure. More intriguingly, successful treatment with captopril was associated with a significant two-fold increase in lymphocyte Gs levels.

Table A14.13a
Signaling abnormalities and Disease

Pseudohypoparathyroidism	$G\alpha_s$
McCune-Albright Syndrome	$G\alpha_s$
Acromegaly	$G\alpha_s$

Hyperfunctional thyroid nodules	$G\alpha_s$
Ovarian and adrenocortical tumors	$G\alpha_{i2}$
Congenital Stationary night blindness	$G\alpha_{t1}$
Right ventricular outflow tachycardia	$G\alpha_{i2}$
Animal model of ulcerative colitis	$G\alpha_{i2}$
Hypertension	$G\beta$
Drosophila learning and memory	Adenylate cyclase
Drosophila learning and memory	PKC
Drosophila learning and memory	CREB
Heart failure	β ARK
Drosophila retinal degeneration	Arrestin
Amphetamine administration	RGS proteins
Circadian arrhythmicity	Transcription factors (clock genes)

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