Physiological Roles of G Protein–Coupled Receptor Kinases and Arrestins

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Abstract

Heterotrimeric G protein–coupled receptors (GPCRs) are found on the surface of all cells of multicellular organisms and are major mediators of intercellular communication. More than 800 distinct GPCRs are present in the human genome, and individual receptor subtypes respond to hormones, neurotransmitters, chemokines, odorants, or tastants. GPCRs represent the most widely targeted pharmacological protein class. Because drugs that target GPCRs often engage receptor regulatory mechanisms that limit drug effectiveness, particularly in chronic treatment, there is great interest in understanding how GPCRs are regulated, as a basis for designing therapeutic drugs that evade this regulation. The major GPCR regulatory pathway involves phosphorylation of activated receptors by G protein–coupled receptor kinases (GRKs), followed by binding of arrestin proteins, which prevent receptors from activating downstream heterotrimeric G protein pathways while allowing activation of arrestin-dependent signaling pathways. Although the general mechanisms of GRK-arrestin regulation have been well explored in model cell systems and with purified proteins, much less is known about the role of GRK-arrestin regulation of receptors in physiological and pathophysiological settings. This review focuses on the physiological functions and potential pathophysiological roles of GRKs and arrestins in human disorders as well as on recent studies using knockout and transgenic mice to explore the role of GRK-arrestin regulation of GPCRs in vivo.

MECHANISMS OF GPCR SIGNALING AND DESENSITIZATION

G protein–coupled receptors (GPCRs) are the largest superfamily of cell surface receptor proteins (1). When the appropriate agonist ligand binds to a receptor on the cell surface, the intracellular domain of the receptor changes conformation in ways that are still poorly understood and results in a conformation that acts as a guanine nucleotide exchange factor for heterotrimeric guanine nucleotide–binding proteins (G proteins). Activated receptors facilitate GDP release from inactive G protein heterotrimers as well as GTP binding to activate the G protein. The receptors derive their name from this common mechanism. Nevertheless, it has become clear in recent years that these receptors do not signal exclusively via heterotrimeric G proteins, although that is a major part of their function. GPCRs are also called seven-transmembrane-span (7TM) or heptahelical receptors, referring to the overall structural motif shared by all three mammalian families of these receptors. Receptors within each of these three families share extensive primary sequence similarity, although the families are very distinct from each other.

GPCRs regulate some part of nearly all physiological functions. There are more than 800 known GPCRs in the human genome. Ligands for these receptors include large glycoprotein hormones, a multitude of peptides, bioactive lipids, amino acids and amino acid metabolites such as dopamine and norepinephrine, small molecules such as acetylcholine and sucrose, calcium ions, and even photons. Despite this great diversity of ligand types, receptor function is generally very modular: Receptors couple to a subset of the 16 heterotrimeric G protein subtypes, which are functionally grouped into four broad classes: G_s, G_i, G_q, and G_{12}. These G proteins in turn regulate a relatively small number of intracellular G protein effectors. Within this system, some receptors couple primarily to one G protein subtype and one effector (G_s activation of adenylyl cyclase, for instance), whereas others promiscuously activate members of all four G protein subtypes and couple to correspondingly more intracellular signaling pathways. GPCRs also perform isotype-specific functions through unique interaction partners and can activate signaling pathways independent of (or parallel to) those mediated by heterotrimeric G proteins. Understanding these unique functions has been of great interest in recent years and has been reviewed extensively (2, 3).

All GPCR systems exhibit context-dependent activity (4): That is, receptor sensitivity changes depending on the amount of signaling that a particular receptor has stimulated on a given cell, so that in general receptors adjust their sensitivity to the range of agonist concentrations to which they are exposed. Receptors desensitize to prolonged or repeated exposure to high agonist concentration and resensitize when not exposed to agonist for some time. One important mechanism for regulating GPCR responsiveness is the G protein–coupled receptor kinase (GRK)-arrestin pathway (**Figure 1**) (5–9). Just as G proteins recognize activated receptors, the GRKs also recognize activated GPCRs, which leads to catalytic activation of the protein kinase and results in receptor phosphorylation at specific sites on the intracellular loops and carboxyl-terminal tail. Because of this recognition, GRKs strongly prefer the activated receptor as substrate. Once phosphorylated, receptors become substrates for binding of arrestin proteins, which prevent the receptor from activating additional G proteins. GRK phosphorylation and arrestin binding result in a cessation of G protein signaling, even despite the continued presence of the receptor-activating agonist. The GRK-arrestin pathway also performs other functions, such as facilitating receptor internalization from the cell surface through clathrin-coated pits. By binding to additional signaling proteins, GRKs and arrestins also function as switches, converting receptor activation from

G protein–coupled receptors (GPCRs): a family of seven-transmembrane proteins that transduce extracellular signals from agonist binding into intracellular signaling pathways

G proteins (guanine nucleotide–binding proteins): a family of proteins that use the binding of guanosine triphosphate (GTP) and its hydrolysis to guanosine diphosphate (GDP) as a molecular switch to transmit biological signals from cell surface receptors to effector enzymes inside the cell

Arrestins: adaptor proteins that bind to GRK-phosphorylated GPCRs to block G protein activation, initiate receptor endocytosis and trafficking, and promote G protein–independent signaling through associated signaling molecules

G protein–coupled receptor kinases (GRKs): protein kinases that recognize and phosphorylate activated GPCRs to promote arrestin binding

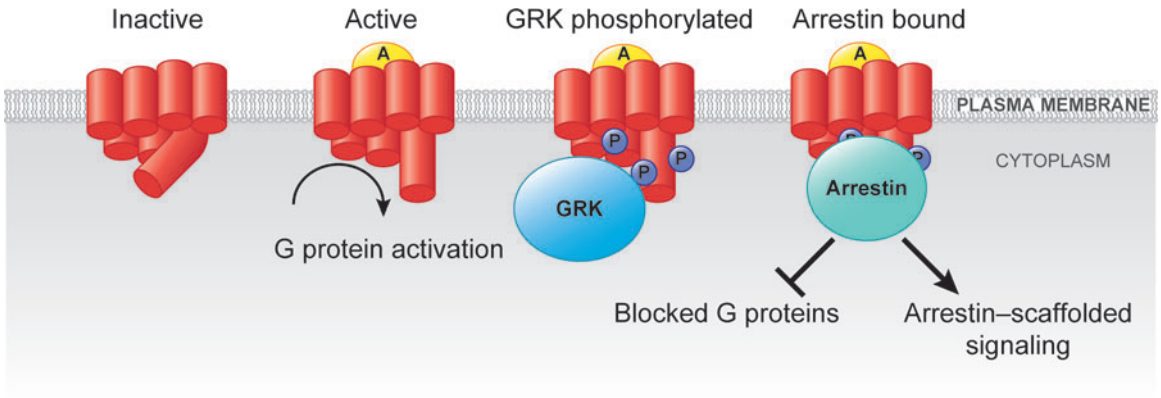

Figure 1

In the absence of an activating agonist ligand (A), the 7TM G protein–coupled receptor (GPCR) on the cell surface is in an inactive conformation and does not activate downstream G proteins or other signaling pathways. When an appropriate agonist binds to the extracellular face of its receptor, the receptor undergoes a conformational change to expose surfaces that act as a guanine nucleotide exchange factor for heterotrimeric G proteins. The activated receptor facilitates release of tightly bound GDP from an inactive G protein bound to the receptor; the receptor then facilitates binding of GTP to the G protein. The GTP-bound G protein undergoes a conformational change that causes its release from the receptor and dissociation into activated α- and βγ-subunits, which each go on to activate downstream effectors (*not shown*). As long as the agonist remains bound to the receptor, this activated receptor can continue to activate G proteins. A G protein–coupled receptor kinase (GRK), which is catalytically activated by this interaction, also recognizes the activated conformation of the receptor. Activated GRKs phosphorylate (P) intracellular domains of the receptor and are then released. The agonist-activated, GRK-phosphorylated receptor binds tightly to an arrestin protein, which interdicts (desensitizes) further G protein activation and couples the receptor to the clathrin-coated-pit internalization pathway and to arrestin-scaffolded (and G protein–independent) signaling pathways.

heterotrimeric G protein signaling pathways to G protein–independent pathways. These pathways have been reviewed extensively (2, 3, 10).

There are seven GRKs in humans, named GRK1 through GRK7, and four arrestin proteins, named arrestins 1 through 4. The GRKs are functionally divided into three classes: GRK1-like, GRK2-like, and GRK4-like. GRK1 (rhodopsin kinase) and the related GRK7 (iodopsin kinase) are primarily found in the retina and regulate the light receptors, the opsins. GRK2 and the related GRK3 are widely expressed, although GRK2 is present at higher levels in tissues, and share a carboxyl-terminal pleckstrin homology domain that mediates PIP_2 and G protein βγ-subunit-mediated translocation of these kinases to the inner leaflet of the plasma membrane near activated receptor substrates. GRK4, GRK5, and GRK6 lack this G protein βγ-subunit binding domain but use direct PIP_2 binding and/or covalent lipid modification with palmitate to reside primarily at the plasma membrane. GRK4 has a limited tissue distribution—it is mainly found in testis—whereas GRK5 and GRK6 are widely expressed (11). Thus, most receptors in the body are potentially regulated by only four of these kinases: GRKs 2, 3, 5, or 6. Arrestins also have visual-specific isoforms, arrestin-1 and arrestin-4, as well as widely expressed somatic isoforms, arrestin-2 (β-arrestin1) and arrestin-3 (β-arrestin2). Likewise, most receptors in the body are subject to regulation only by β-arrestin1 or -2.

PIP_2: phosphatidyl inositol bisphosphate

Most of what has been learned about receptor regulation and signaling via GRK-arrestin pathways has been determined using model cell systems (2, 3, 5–8). Although

Table 1 Major phenotypes in mice deficient in nonvisual GRKs and arrestins

GRK or arrestin	Knockout phenotype
GRK2 [β-adrenergic receptor kinase (βARK), βARK1]	Embryonic lethal, thin myocardium syndrome in embryos (19); enhanced basal and adrenergic responses in cardiac function in adult heterozygotes (18); altered progression of experimental autoimmune encephalomyelitis (48)
GRK3 (βARK2)	Lack of olfactory receptor desensitization (82); altered M2 muscarinic airway regulation (20); blunted kappa-opioid receptor–mediated tolerance in spinal analgesia test (90, 91); disrupted tolerance to the antinociceptive effects of fentanyl but not morphine (89).
GRK4 (IT11)	Normal fertility and sperm function (R.T. Premont, unpublished data). No obvious phenotype.
GRK5	Altered central (96) and lung (51) M2 muscarinic receptor regulation, with normal heart M2 receptor regulation (51).
GRK6	Altered central dopamine receptor regulation (99, 102); deficient lymphocyte chemotaxis (44); increased acute inflammation and neutrophil chemotaxis (45, 46).
β-arrestin1 (arrestin-2)	Altered cardiac responses to beta-adrenergic stimulation (21).
β-arrestin2 (arrestin-3)	Enhanced morphine antinociception (115, 118), reward (125), disrupted morphine tolerance (116), and reduced constipation and respiratory suppression (120); reduced locomotor activity and disrupted dopamine-mediated behaviors (127); deficient lymphocyte chemotaxis (44); altered susceptibility to endotoxic shock and expression of proinflammatory cytokines (49); altered CXCR2-mediated neutrophil chemotaxis (50); altered asthmatic response to allergens (52); decreased bone mass and altered bone architecture (56, 57).

the pathways appear very interesting, it is often unclear whether they actually function in any specific organ in a living organism or have detectable physiological consequences. Studies using knockout and transgenic mice, studies using viral-mediated overexpression or short hairpin RNA (shRNA) knockdown, and genetic studies in human patients recently have begun to probe the physiological roles of GRKs and arrestins. These studies confirm that GRKs and arrestins do regulate GPCR pathways in humans and other animals with a specificity that is only dimly understood and that altering these regulatory pathways can have profound influences on physiological responses and pathophysiology. Owing to the nature of GRK function, involving the regulation of activated receptors, mice lacking GRKs or arrestins often look completely normal until stressed by exposure to a receptor activator. Likewise, because the GRK-arrestin system can have dual roles, those of both suppressing G protein signaling and promoting non–G protein signaling, loss of this regulation can have two opposing effects on physiological systems: (*a*) allowing enhanced or unregulated receptor signaling through loss of desensitization or (*b*) decreasing signaling by preventing the switch from G protein to non–G protein pathways.

shRNA: short hairpin RNA

This chapter reviews the role of nonvisual GRKs and arrestins in a few physiological systems to outline what has been learned so far and how much remains to be explored. **Table 1** summarizes the characterized phenotypes of GRK and β-arrestin knockout mice. Because the large literature on rhodopsin regulation by GRK1/rhodopsin kinase and arrestin regulation in the visual system has been reviewed recently (12–14), we do not discuss this here.

GRKs AND ARRESTINS IN THE CARDIOVASCULAR SYSTEM

GPCRs and their agonists play important roles throughout the cardiovascular system. Blood pressure is controlled by altering heart rate, vascular resistance, and fluid/electrolyte balance, each of which is regulated to a large degree by GPCRs. Heart rate is regulated by adrenergic and muscarinic receptor

activation by norepinephrine and acetylcholine, respectively. Vascular tone is regulated by angiotensin II and bradykinin receptor activation, and kidney water and salt reabsorption (fluid balance) is regulated by angiotensin II, vasopressin, and dopamine receptor activation. Because GPCRs play such diverse and fundamental roles in regulating multiple aspects of cardiovascular function, GRKs and arrestins are important in controlling the sensitivity and responses of these organs.

Researchers have studied in detail the GRK regulation of direct cardiac function, using cardiomyocyte overexpression of GRKs and GRK fragments (i.e., GRK2 carboxy terminus as a G protein βγ-subunit scavenger). As this area has been reviewed extensively in recent years (15–17), we do not discuss these transgenic overexpression studies further here. Overall, there is good evidence that GRK2 is important for regulating several cardiac receptor responses and that GRK3 and GRK5 are involved in some receptor responses but not others. Studies in knockout animals also support the important role of GRKs and β-arrestins in cardiac responses. Genetic deletion of GRK2 in mice results in embryonic lethality owing to hypoplasia of the ventricular myocardium, directly demonstrating a critical role of this kinase in cardiac development, whereas GRK2-heterozygous mice exhibit altered cardiac function (18, 19). GRK3 knockout mice also showed altered cardiovascular responses to cholinergic stimulation (20). Deletion of β-arrestin1 in mice does not change resting cardiovascular parameters but results in altered cardiac responses to β-adrenergic stimulation (21).

More recent work has expanded into other aspects of cardiovascular regulation by focusing on overexpression of GRK2 and GRK5 in vascular smooth muscle cells. Hypertension has been associated with altered GRK expression and function. Human patients with hypertension have increased GRK2 expression in lymphocytes (22, 23), and hypertensive rats have increased vascular smooth muscle cell expression of GRK2 (24). Eckhart and colleagues (25) found that mice overexpressing GRK2 from the smooth muscle–specific SM22α promoter had elevated resting blood pressure. These mice also exhibited reduced sensitivity to vascular muscle relaxation by the β-adrenergic agonist isoproterenol and to increased mean arterial blood pressure in response to the vasoconstrictive peptide angiotensin II. SM22α-GRK2-overexpressing mice had a greatly increased smooth muscle cell layer thickness in the aortic medial layer, suggested to result from prolonged exposure to elevated blood pressure. Increased cardiac hypertrophy was also evident, despite normal GRK2 expression in the cardiomyocytes (25). In contrast, although SM22α-GRK5 mice were also hypertensive, they did not exhibit either aortic or cardiac hypertrophy (26). Interestingly, male and female mice overexpressing GRK5 displayed distinctly different mean levels of hypertension; male mice had the highest mean blood pressure. Systemic treatment of the mice with pertussis toxin to inactivate the heterotrimeric G_i proteins normalized the blood pressure, suggesting that G_i-linked receptor pathways are affected by the extra GRK5 (26). Both β-adrenergic and angiotensin pathways are altered, but to differing extents in male and female mice. In total, these studies validate the role of GRKs in regulating vascular tone and responsiveness through the regulation of smooth muscle cell GPCRs. Defining the specificity of vascular receptor–GRK coupling and identifying the precise pathways that are altered to lead to hypertrophy and/or increased blood pressure will require further studies.

Dopamine D1 receptors in the kidney increase sodium (and water) excretion in response to an increased sodium load (27). Essential hypertension in humans has been associated with decreased coupling of dopamine stimulation to sodium excretion (28). Polymorphisms in the human GRK4 gene appear to account for some of this variation. Studies with kidney tubule cells isolated from hypertensive and normotensive humans suggest

eNOS: endothelial nitric oxide synthase

that constitutive activity of some polymorphic variants of the GRK4 protein leads to chronically phosphorylated, inactive D1 receptors (29). This model finds support in (*a*) studies in model cells in which transfected GRK4 variants display marked constitutive kinase activity toward the D1 dopamine receptor (30) as well as in (*b*) a study, in rats, in which GRK4 antisense treatment of kidney tubules in hypertensive rats normalized sodium excretion, urine volume, and blood pressure (31). Various recent studies of hypertensive human populations find a positive correlation between certain GRK4 polymorphisms, or haplotypes, and hypertensive disease (32–34). Thus, hypertension-associated polymorphic variants of GRK4 appear hyperactive, leading to continuous kidney dopamine receptor desensitization and loss of dopamine-stimulated salt and fluid excretion.

GRK2 has a quite unexpected role in the liver, where it is an important regulator of portal blood pressure. Mice with only one active copy of the GRK2 gene are relatively resistant to liver injury–induced portal hypertension (35). Using rats injured with bile duct ligation, Liu et al. (35) found that GRK2 levels increased in injured liver endothelial cells. Previous work had shown that endothelin receptors stimulate the Akt protein kinase to phosphorylate endothelial nitric oxide synthase (eNOS) through a G protein βγ-subunit-activated, phosphatidylinositol 3-kinase–dependent mechanism, increasing nitric oxide production (36). In such a pathway, GRK2 would normally be expected to act to phosphorylate and desensitize the endothelin receptors, leading to reduced signaling to downstream Akt and eNOS and thus to reduced nitric oxide production. However, in endothelial cells from rats injured by bile duct ligation, concomitant with the increased level of GRK2 protein is an increased direct association of GRK2 with Akt (35). GRK2 bound to Akt inhibits the kinase activity of Akt, leading to reduced eNOS phosphorylation and reduced nitric oxide release (35). Adenoviral overexpression of GRK2 leads to increased portal pressure in otherwise normal rats, and the injury-induced deficit in Akt activation can be circumvented by expressing activated Akt in the liver portal system after infection with a recombinant adenovirus (35). Thus, GRK2 acts in an apparently noncatalytic manner to reduce the signaling of Akt. Undoubtedly, this GRK2-mediated inhibition of Akt plays important roles in other tissues as well, and the prevalence of GRK2 expression level changes under pathophysiological conditions indicates that GRK2-mediated inhibition of Akt may be prominent in a variety of disease states.

GRKs AND ARRESTINS IN THE IMMUNE SYSTEM

GPCRs have myriad roles within the immune system. The CC and CXC chemokine peptides (SDF-1, RANTES, and many others) and most arachidonic acid metabolites (prostaglandins, eicosinoids, leukotrienes) activate GPCRs, as do complement (C3a, C5a) and bacterial-derived products (fMLP). As such, G protein pathways are critical to immune cell chemotaxis, homing, activation, and target tissue recruitment of immune cells.

Cells of the immune system have particularly high expression of GRK2 and GRK6 (37, 38). Furthermore, altered expression of GRK2 and GRK6 occurs in immune cells isolated from human patients with a variety of immunological and other diseases as well as in animal models of disease. For example, lymphocytes from humans with rheumatoid arthritis have decreased GRK2 and GRK6 expression, and treating normal lymphocytes with the proinflammatory interferon-γ or interleukin 6 leads to decreases in GRK2 expression (39). Cultured human T lymphocytes exposed to oxidative stress with H_2O_2 or by coculture with activated neutrophils have reduced GRK2 expression (40). Induction of acute adjuvant arthritis in rats results in a reduction in lymphocyte GRK2 and GRK6 levels (41), as does induction of allergic encephalomyelitis (42). Humans with hypertension have increased GRK2

expression in lymphocytes (22, 23). Hypertensive rats have increased lymphocyte GRK2 expression (24). Chronic injection of rats with the β-adrenergic receptor agonist isoproterenol leads to increased expression of GRK2 and GRK5 mRNA in lymphocytes (43). Thus, leukocyte GRK levels are subject to regulation by a wide variety of factors and disease states.

Studies using mice lacking GRK6 or β-arrestin2 have revealed altered regulation of immune cells. In lymphocytes, ablation of GRK6 or β-arrestin2 leads to augmented signaling by SDF-1 (CXCL12) acting through the CXCR4 receptor (44). This increased signaling is associated with a reduced ability of T lymphocytes to chemotax through a filter or through an endothelial cell barrier toward SDF-1 (44). This decreased chemotaxis may be due to either overactive (undesensitized) traditional G protein signaling pathways or decreased β-arrestin-mediated signaling events.

In neutrophils, ablation of GRK6 leads to augmented signaling by leukotriene B4 (LTB4) acting through the BLT1 receptor; however, these receptors do continue to desensitize following repeated LTB4 administration (45). Neutrophils isolated from GRK6 knockout mice chemotax more robustly toward LTB4 and display a higher basal migration activity (45). Arachidonic acid applied to the ear is converted to LTB4 and other active metabolites and stimulates a rapid inflammatory response with both neutrophil influx and edema. GRK6 knockout and -heterozygous mice exhibit an enhanced response characterized by greater tissue swelling and by increased neutrophil infiltration into the tissue, consistent with increased chemotaxis toward LTB4 (45).

Neutrophils also respond to SDF-1 acting through CXCR4 receptors on those cells; this pathway is important in facilitating neutrophil retention in the bone marrow. Neutrophils mobilize to the blood in response to granulocyte colony–stimulating factor (G-CSF), in part by overcoming CXCR4-mediated retention signals. Mice lacking the GRK6 gene are less able to mobilize neutrophils from bone marrow into the blood in response to G-CSF (46). This reduced mobilization appears to result from an inability of G-CSF to overcome SDF-1/CXCR4-mediated retention signals, as neutrophils from GRK6 knockout mice chemotax more robustly to SDF-1 than do wild-type cells, either before or after G-CSF treatment of the mice (46). Calcium influx signaling downstream of CXCR4 fails to desensitize in neutrophils from GRK6 knockout mice, in contrast to what occurs in wild-type cells (46).

Studies using GRK2 knockout mice have also shown that GRK2 is important in immune cells. Because the full two-allele knockout of GRK2 is embryonic lethal (19), in vivo studies have instead focused on heterozygous mice bearing only one active GRK2 gene. In GRK2-heterozygous mice, T lymphocytes display increased chemotaxis toward the CCR5 receptor ligands CCL4 and CCL5 and toward the CCR1 ligand CCL3 (47). The CCR5 receptor exhibits reduced phosphorylation in lymphocytes with reduced GRK2, but enhanced signaling, consistent with a decrease in receptor desensitization (47). GRK2-heterozygous mice also display a marked sensitivity to experimental autoimmune encephalomyelitis, a model for multiple sclerosis, displaying a more rapid onset of lymphoctye infiltration into the brain (48). Despite the more rapid onset, the GRK2-heterozygous mice, unlike wild-type mice, fail to undergo relapse. Thus, decreased GRK2 can have both deleterious and protective effects in this disease model. More complete investigation of the role of GRK2 awaits the availability of mice bearing a conditional GRK2 allele.

Recently, an essential role of β-arrestin2 as a negative regulator of innate immune activation via Toll-like receptor–interleukin 1 receptor signaling was indicated by demonstration of higher expression of proinflammatory cytokines and increased susceptibility to endotoxic shock of β-arrestin2 knockout mice (49). Furthermore, an altered neutrophil

Arrestin-mediated signaling: the use of arrestin proteins as adaptors carrying signaling molecules to phosphorylated receptors to initiate receptor-dependent, G protein–independent signals

LTB4: leukotriene B4

G-CSF: granulocyte colony–stimulating factor

CXCR2 signaling in vivo (50) and defective lymphocyte chemotaxis were also described in mice lacking β-arrestin2 (44).

GPCR desensitization: the loss of cellular responsiveness to repeated or prolonged receptor stimulation; tachyphylaxis

CNS: central nervous system

GRKs AND ARRESTINS IN THE RESPIRATORY SYSTEM

Contraction and relaxation of airway smooth muscles are controlled, at least in part, by multiple GPCRs, and dysfunctional GPCR-mediated signaling has been implicated in asthma. Researchers have investigated the role of GRKs and β-arrestins in airway smooth muscle physiology. Mice lacking GRK3, but not GRK2-heterozygous mice, demonstrate a significant enhancement in the airway response and enhanced sensitivity of the airway smooth muscle response to cholinergic agonist methacholine, indicating that GRK3 may be involved in modulating the cholinergic response of airway smooth muscle (20). GRK5 is also involved in muscarinic regulation of airway responses (51). Although the airway contractile response to a muscarinic receptor agonist was not altered in GRK5-deficient mice, the relaxation component of bilateral vagal stimulation and the airway smooth muscle relaxation resulting from adrenergic stimulation were diminished in GRK5 mutants. Intriguing recent observations also indicate that β-arrestin2 may be involved in the development of allergic asthma (52). Allergen-sensitized mutant mice lacking β-arrestin2 do not accumulate T lymphocytes in their airways and do not demonstrate physiological and inflammatory features of asthma, suggesting that targeting processes controlled by this regulatory protein will provide novel insights in the treatment of asthma.

GRKs AND ARRESTINS IN THE SKELETAL SYSTEM

GRK2 and β-arrestin1 are expressed in osteoblastic cells (53). Transgenic mice expressing the GRK2 carboxyl-terminal inhibitor in mature osteoblasts demonstrated age-dependent enhancement in bone remodeling, as evidenced by an increase in bone density and trabecular bone volume (54, 55). In contrast, mice lacking β-arrestin2 showed decreased bone mass and altered bone architecture (56), revealing an important role of this protein in parathyroid hormone–dependent bone mass acquisition and remodeling (57).

GRKs AND ARRESTINS IN THE NERVOUS SYSTEM

More than 90% of known GPCRs are expressed in the brain (58) and are involved in virtually all vital functions controlled by the nervous system. It is not surprising, therefore, that most of the research on the physiological relevance of GPCR desensitization mechanisms has been performed in analyzing neuronal functions (9). Although sensory GPCRs of the olfactory and gustatory systems potentially are subject to regulation by GRKs and arrestins, only a few such studies have been published to date (58a, 82). As the most extensive evidence for wide variety of physiological functions and distinct tissue distributions of GRKs and arrestins comes from studies of the central nervous system (CNS), here we give a detailed overview of these observations in a more systematic manner by highlighting specific roles of individual neuronal GRKs and arrestins.

Neuronal GRK2

The GRK2 kinase has widespread expression in the brain (59). GRK2 mRNA is distributed in a nearly uniform manner through all cortical layers, the islands of Calleja, several hypothalamic and thalamic nuclei, the hippocampus, the substantia nigra compacta, the ventral tegmental area, the locus coeruleus, and other regions. A lower level of expression was detected in caudate-putamen (60). The expression of GRK2 is developmentally regulated, with a marked increase during the second postpartum week (61).

Alterations in GRK2 expression have been described in several disorders and/or

following pharmacological treatments. In patients with major depression, there is an upregulation of GRK2-like immunoreactivity in the prefrontal cortex, whereas long-term antidepressant treatment downregulates GRK2 (62). One interesting hypothesis suggests that the neuronal calcium sensor-1 (NCS-1) contributes to the desensitization of D2 dopamine receptors via interaction with GRK2, thereby attributing abnormalities in NCS-1 expression described in schizophrenia and bipolar disorder to the NCS-1-dependent GRK2 regulation of dopamine receptor signaling (63, 64). Furthermore, GRK2 is increased in caudal caudate and internal globus pallidus in monkeys with experimental parkinsonism, indicating a potential role of this kinase in regulation of dopamine receptors (65). Mice heterozygous for GRK2 deletion were tested for their locomotor responses to the dopamine transporter blocker cocaine or the direct dopamine receptor agonist apomorphine. Cocaine treatment with certain doses resulted in slightly enhanced locomotor responses, whereas no such alterations were found with other doses of cocaine or with climbing responses to the nonselective dopamine agonist apomorphine, suggesting that the impact of partial loss of GRK2 on dopamine receptor–mediated responses is limited (9). Nevertheless, a more pronounced level of GRK2 deficiency may unmask the involvement of this kinase in dopamine receptor regulation. Further studies involving region-selective knockout mice may provide an effective approach with which to examine this possibility.

Several lines of evidence suggest a role for GRK2 in μ-opioid (μOR) regulation. GRK2 levels were increased in the locus coeruleus and cortex of rats chronically treated with morphine (66–68). Similarly, membrane-associated GRK2 levels were increased in brains of human opioid addicts (67). In another investigation, chronic treatment with the opioid antagonist naltrexone resulted in significant upregulation of GRK2 (69). Recent evidence indicates that chronic treatment with etorphine, but not morphine, produces a significant increase in GRK2 protein levels in membranes of the mouse spinal cord (70). In addition, GRK2 is highly expressed in nucleus raphe magnus GABAergic neurons projecting to the spinal cord, where it appears to mediate desensitization of μOR (71). These and other (72, 73) findings suggest that GRK2 contributes to the cellular processes underlying in vivo μOR desensitization and may play an important role in the development of opioid tolerance and dependence. A preliminary investigation, however, found no significant alteration in morphine-induced analgesia in GRK2-heterozygous mice (74). Further studies are necessary to explore in more detail the role of GRK2-mediated processes in μOR regulation and responses to opiates.

The α and β isoforms of synucleins, proteins highly expressed in the brain and linked to the development of Parkinson's and Alzheimer's diseases, can be potently phosphorylated by GRK2 and GRK5 (75). Abnormalities in GRK2/5-like immunoreactivity associated with beta-amyloid accumulation were observed at prodromal and early stages of Alzheimer's disease (76). In neonatal rat, hypoxia/ischemia reduced GRK2 expression in several brain regions, suggesting a role of this kinase in hypoxia-induced brain damage (77). GRK2 is involved in both phosphorylation-dependent and -independent regulation of M1 muscarinic acetylcholine receptors in cultured hippocampal neurons (78). Additionally, GRK2 may have a role in the regulation of corticotropin-releasing factor receptor type 1 in the anterior pituitary gland (79).

Neuronal GRK3

GRK3 is expressed widely in the periphery and the CNS, albeit at lower levels than is GRK2 (59). In the periphery, GRK3 is found in olfactory neurons and dorsal root ganglion neurons, where it may play an important role in the desensitization of odorant receptors and α_2-adrenergic receptors, respectively (80–82).

NCS-1: neuronal calcium sensor 1 protein

GABA: γ-amino butyric acid

μOR: μ-opioid receptor

5HT: 5-hydroxytryptamine, or serotonin

GRK3 is ubiquitously expressed in the brain in a pattern similar to GRK2 (59, 60).

Substantial in vitro evidence suggests that GRK3 has a role in dopamine receptor regulation (9). Interestingly, the region of chromosome 22q12 containing the GRK3 gene has been identified as a susceptibility locus for bipolar disorder (83). Furthermore, GRK3 expression in the rat frontal cortex can be induced by the dopamine releaser amphetamine (83). Additionally, transmission disequilibrium analyses indicate that two 5′-UTR/promoter polymorphisms may be associated with bipolar disorder (83). These findings were interpreted to suggest that a dysregulation in GRK3 expression alters dopamine receptor desensitization and thereby predisposes affected individuals for this disorder (83, 84). Recent assessment of GRK3 mRNA levels in lymphocytes from bipolar patients, however, did not reveal a major difference between patients and controls (85). Similarly, mutation screening and association study of GRK3 in schizophrenia families provided no evidence for an association between schizophrenia and alleles at polymorphisms in the GRK3 promoter region (86).

In mice, GRK3 deficiency does not affect basal locomotor activity, but GRK3 knockout mice demonstrate significantly reduced locomotor or climbing responses to either cocaine or apomorphine (9). Thus, it is unlikely that this kinase is directly involved in the desensitization of dopamine receptors responsible for locomotion. Nevertheless, it is possible that GRK3 is "positively" involved in dopamine receptor signaling leading to locomotor responses or "negatively" regulates other populations of GPCRs, such as, for example, receptors for 5HT (serotonin), that can inhibit dopamine-related behaviors (87). According to another interesting hypothesis, this kinase may be involved in the desensitization of D3 dopamine autoreceptors, thereby affecting processes governing dopamine release (88).

A role for GRK3 in μOR regulation also has been suggested on the basis of altered expression of this kinase following the administration of opiate agonists and antagonists (68, 69). Other researchers, however, failed to observe such changes following chronic morphine (66). In GRK3 knockout mice, morphine-induced analgesia was not changed in a hot-plate analgesia test (74). At the same time, GRK3 deletion influenced opioid analgesic tolerance to a high-efficacy opioid agonist, fentanyl, but did not affect acute antinociceptive responses to either fentanyl or morphine (89). Furthermore, there is evidence suggesting that GRK3 is involved in kappa-opioid receptor regulation. Neuropathic pain–induced activation of the kappa-opioid system in mouse spinal cord and kappa-opioid receptor–dependent tolerance are disrupted in GRK3 knockout mice (90, 91).

Neuronal GRK4

Expression of GRK4 in the brain is limited to cerebellar Purkinje cells (92). GRK4 may regulate the cerebellar metabotropic glutamate 1 receptor and GABA-B receptor (93, 94), suggesting a role of GRK4 in motor coordination and learning. However, mice lacking GRK4 demonstrate no differences in basal level of locomotor activity or motor coordination in a rotorod test (R.T. Premont, unpublished data). GRK4-deficient and control mice also have similar locomotor responses to cocaine (9). Furthermore, no alterations in morphine-induced analgesia have been found in these mutants (74).

Neuronal GRK5

GRK5 is expressed widely in the brain. GRK5 mRNA has been found at high levels in various regions, such as the septum, the cingulate cortex, the septohippocampal nucleus, the anterior thalamic nuclei, the medial habenula, and the locus coeruleus (60). GRK5 expression increases twofold during neural differentiation (95).

Chronic treatment with cocaine resulted in upregulation of GRK5 mRNA in the

septum, suggesting a role of this kinase in abnormal plasticity induced by this drug (60). Similarly, an acute administration of morphine, as well as spontaneous and naloxone-induced morphine withdrawal, caused up-regulation of GRK5 mRNA in some brain regions (72). GRK5 deficit may play a role in prodromal and early stages of Alzheimer's disease (76).

Mice lacking GRK5 have an overtly normal phenotype and show only a slight decrease in body temperature (96). Mutant mice were challenged with a number of agonists to identify the specific GPCRs affected by GRK5 deficiency (9, 96). We observed no differences in cocaine-induced stimulation of locomotion or climbing responses following administration of the direct dopamine agonist apomorphine (9, 96). Similarly, morphine-induced analgesia was not altered in a hot-plate test in mutant mice. Furthermore, hypothermic responses induced by serotonin 5-HT1A receptor agonist 8-OH-DPAT did not differ between the genotypes. These observations indicate that responsiveness of dopamine receptors, and of the μOR and 5-HT1A subtype of serotonin receptors relevant for these behavioral and physiological processes, was not affected by deletion of GRK5. However, challenging these mice with the nonselective muscarinic agonist oxotremorine revealed significantly enhanced, centrally mediated muscarinic responses such as hypothermia, tremor, salivation, locomotor suppression, and antinociception (96). Because M2 muscarinic receptors mediate most of these behaviors (97), GRK5 likely regulates M2 muscarinic receptors in vivo. Furthermore, recent studies revealed that M2 receptors in airway are also affected by GRK5 deficiency, whereas regulation of M2 receptors in the heart occurs normally (51). These observations demonstrate that the same GRK can regulate the same GPCR differently in different tissues. Moreover, the hypersalivation response to oxotremorine displayed by the GRK5 mutants suggests that GRK5 also regulates salivary gland M3 receptors in vivo (97). Supersensitivity of central muscarinic receptors may contribute to several brain disorders, including depression, posttraumatic stress disorder, and multiple chemical sensitivities. It would be interesting to explore if abnormalities in GRK5-mediated muscarinic receptor desensitization contribute to these disorders (96).

Neuronal GRK6

GRK6 is ubiquitously expressed in most brain areas studied (98). Interestingly, GRK6 seems to be the most prominent GRK in the caudate putamen, and GRK6 mRNA is also expressed in dopaminergic cell body areas, such as the substantia nigra (60). In striatum, immunohistochemical investigations revealed a high expression of GRK6 protein in the dopamine-receptive striatal GABAergic medium spiny neurons as well as in cholinergic interneurons (99).

Unlike all other GRK-deficient mice, GRK6 knockout mice show significant locomotor supersensitivity to cocaine, amphetamine, and morphine (99). Similar effects were observed with endogenous "trace amine" β-phenylethylamine, which induces psychomotor activation at least partially via indirect activation of the dopamine system (100, 101). Furthermore, researchers observed in mutants (*a*) that an enhanced coupling of striatal D2-like dopamine receptors to G proteins increased the affinity of D2 but not D1 dopamine agonists and (*b*) an enhanced locomotor response to direct dopamine agonists (99, 102). Altogether, these observations indicate that postsynaptic D2-like dopamine receptors in the striatum are physiological targets for GRK6-mediated regulation, suggesting that a pharmacological strategy aimed at modulation of GRK6 expression or activity is beneficial when dopamine signaling is altered, such as in Parkinson's disorder. In fact, GRK6 expression is significantly elevated in several brain regions, including the striatum, in the MPTP-lesioned monkey model of Parkinson's disease (65). Moreover, because dopamine

receptor supersensitivity is present in other brain disorders, such as addiction, schizophrenia, and Tourette's syndrome, a potential role of GRK6 in these conditions should be investigated (99).

GRK6 levels were changed in rat brain after chronic treatment with μOR agonists and antagonists (68, 69). Furthermore, decreased immunodensities of GRK6, GRK2, and β-arrestin2 were observed in postmortem brains of opiate addicts (103). Nonetheless, mice lacking GRK6 were indistinguishable from control littermates in the hot-plate morphine analgesia test (74).

Neuronal β-Arrestin1

β-Arrestin1 is expressed ubiquitously in the brain (9, 61, 95, 104–106). Levels of β-arrestin1 mRNA in the brain are estimated to be two- to threefold higher than those of β-arrestin2 mRNA, whereas the ratio of β-arrestin1 to β-arrestin2 protein levels is 10–20-fold (106). Strong immunoreactivity for β-arrestin1 also has been found at postsynaptic densities in the spinal cord (107). β-Arrestin1 greatly increases with neural development, suggesting a specific role for β-arrestin1 in neural differentiation (95).

Chronic systemic morphine treatment resulted in an increase in β-arrestin1/2-like immunoreactivity in rat locus coeruleus (66). Changes in β-arrestin1 mRNA in several brain areas also were detected following acute and systemic treatment with morphine (108). However, direct testing of acute morphine-induced antinociception in mice lacking β-arrestin1 has not revealed significant changes (74).

β-Arrestin1 levels were reduced in leukocytes of patients with depression and elevated by chronic antidepressants imipramine, desipramine, and fluvoxamine in rat cortex and hippocampus. The reduction in β-arrestin1 levels in the lymphocytes of major depression patients was significantly correlated with the severity of depressive symptoms (109). In addition, cerebral hypoxia/ischemia in rat pups increased β-arrestin1 protein expression as well as mRNA levels in the brain (77).

β-Arrestin1 knockout mice are overtly normal, but locomotor-stimulating effects of cocaine and apomorphine-induced climbing are somewhat reduced (9). These observations suggest that, although β-arrestin1 is not likely involved directly in dopamine receptor desensitization, β-arrestin1 may be involved in either G protein–independent dopamine receptor signaling or desensitization/signaling of other GPCRs in neuronal pathways contributing to the locomotor behaviors.

Neuronal β-Arrestin2

β-Arrestin2, much like β-arrestin1, is expressed in virtually all brain regions and in the spinal cord (9, 95, 104, 106, 107). In many brain tissues there is a significant overlap in the expression pattern of β-arrestin2 and β-arrestin1, but each β-arrestin also has a unique distribution in certain brain areas. In particular, β-arrestin2 has relatively higher expression in the medial habenula, in most hypothalamic nuclei, and in the extended amygdala (106).

Various in vivo studies indicate that β-arrestin2 is involved in μOR regulation and effects of opiates. Several groups have shown that acute or chronic treatment with μOR ligands, including morphine, causes significant alterations in the expression of β-arrestin2 in the cortex and striatum (68, 69, 108). The involvement of β-arrestin2 in the modulation of spinal antinociception also is supported by studies in which intrathecal pretreatment with β-arrestin2 antibody potentiated the antinociception induced by μOR agonists in the mouse (110).

Interestingly, earlier in vitro investigations in heterologous cellular systems found that the interaction of μOR with β-arrestin2 depends on agonist efficacy and that the partial agonist morphine produces arrestin-dependent receptor desensitization at a rather slow rate, suggesting that morphine activates μORs without promoting their rapid

endocytosis (111–113). However, recent studies convincingly demonstrated that, in striatal and nucleus accumbens neurons, morphine promotes rapid, arrestin-dependent endocytosis of μORs (114). In dissociated primary cultures of rat striatal neurons, morphine promoted a rapid redistribution of both endogenous and recombinant μORs (114). These results strongly support previous in vivo results from β-arrestin2 knockout mice (74, 115, 116) and indicate that morphine is indeed capable of driving rapid endocytosis of μORs in vivo and that β-arrestin2 plays an important role in these effects (117).

β-Arrestin2 knockout mice were instrumental to demonstrating the role of β-arrestin2 in the regulation of μORs and effects of morphine. Mutant mice challenged with morphine showed remarkably enhanced antinociception in hot-plate tests, which is correlated with potentiated μOR–G protein coupling (115, 118). Furthermore, tolerance to morphine's antinociceptive effects in this test was significantly attenuated in β-arrestin2 knockout mice (116). At the same time, manifestations of naloxone-precipitated withdrawal were not altered by β-arrestin2 deletion, suggesting that despite disrupted tolerance mutant mice still can become physically dependent on morphine (116). When morphine effects on spinal cord–mediated antinociception were assessed in the tail-immersion test, the β-arrestin2 knockout mice also showed higher basal nociceptive thresholds as well as significantly enhanced responses to morphine. However, mutants developed tolerance to chronic morphine in this test, albeit with a delayed onset, suggesting the additional involvement of protein kinase C (PKC)-dependent regulatory system in this effect (118). A subsequent study in these mutants documented that relative opiate efficacy is determined by the ability to recruit elements of the GPCR desensitization machinery (119). Opiate agonists that induced robust β-arrestin2 translocation in HEK cells produced similar analgesia in normal and β-arrestin2 knockout mice, whereas morphine and heroin, which did not promote robust β-arrestin2 recruitment under these specific conditions, produced enhanced analgesia in mutant mice. Additionally, well-known side effects of morphine, such as constipation and respiratory suppression, were strikingly reduced in β-arrestin2 knockout mice (120), suggesting a novel approach of modulating GPCR regulatory mechanisms to overcome unwanted effects of the gold-standard analgesic, morphine. A recent study employing rats with overexpression of β-arrestin2 at periaqueductal gray (PAG) further supported a role of this regulatory molecule in μOR regulation and morphine antinociception (121). The demonstration that local β-arrestin2 overexpression by adenovirus results in disruption of the antinociceptive effects of morphine clearly identified PAG as a key area for this regulation.

IPSC: inhibitory postsynaptic current

Recently, a role of β-arrestin2 in delta-opioid receptor function was suggested by the observation that delta-opioid receptor agonists had no effect on miniature inhibitory postsynaptic currents (IPSCs) in β-arrestin2 knockout mice after chronic morphine. Thus, induction of delta-opioid receptor–mediated actions in PAG by chronic morphine may require prolonged μOR stimulation and induction of β-arrestin2 expression (122). Furthermore, when the effects of morphine and fentanyl were analyzed in slices of the locus coeruleus and PAG from β-arrestin2 knockout mice, presynaptic inhibition of evoked IPSCs was enhanced. β-Arrestin2 therefore may attenuate presynaptic inhibition by opioids, thereby revealing a novel mechanism of involvement of β-arrestin2 in opioid effects (123).

Finally, β-arrestin2 knockout mice show significant alterations in locomotor and rewarding properties of morphine. The activation of both μOR and dopamine receptors is required for the locomotor and reinforcing effects of morphine (124, 125). Morphine-induced stimulation of dopamine systems is indirect, originating from a disinhibition of GABAergic cells in dopaminergic cell body

regions, leading to increased neuronal firing and increased dopamine release from terminals. In β-arrestin2-deficient mice, morphine, but not cocaine, produces a more pronounced increase in dopamine release and reward in the conditioned place preference test. However, acute morphine induces less locomotor activation in β-arrestin2-mutant mice (125). This paradoxical observation may indicate either (*a*) an important impact of this mutation on neurotransmitter systems other than that of dopamine, perhaps serotonin, that are also involved in morphine effects and exert an inhibitory action on dopamine-dependent hyperactivity (87, 126) or (*b*) a novel positive role of β-arrestin2 in dopaminergic signaling.

In fact, β-arrestin2-deficient mice exhibited somewhat reduced locomotor activation following cocaine administration (125) as well as disrupted climbing response to the direct dopamine agonist apomorphine (9, 127). These in vivo observations are not consistent with an expected role of β-arrestin2 as a controller of desensitization of dopamine receptors but rather indicate that without this regulatory element dopaminergic signaling may be impaired. Recent functional biochemical studies directly demonstrated an important role of β-arrestin2 in D2 dopamine receptor–mediated signaling in vivo (127). D2 dopamine receptor–mediated Akt regulation involves the formation of signaling complexes containing β-arrestin2, protein phosphatase 2A, and Akt. Mice lacking β-arrestin2 show a reduction of amphetamine- and apomorphine-induced behaviors, loss of Akt regulation by dopamine, and disruption of the dopamine-dependent interaction of Akt with PP2A. Furthermore, deletion of β-arrestin2 results in a reduced level of spontaneous locomotor activity in normal as well as hyperdopaminergic dopamine transporter knockout mice (128), directly demonstrating the importance of this regulatory molecule in functional dopaminergic signaling (127). These results for the first time demonstrated in vivo the physiological significance of the dual role of β-arrestin2 in GPCR regulation: In addition to its classical "negative" function in receptor desensitization, β-arrestin2 also acts as a "positive" signaling intermediate as a kinase/phosphatase scaffold. Most importantly, these data indicate that this role of β-arrestin2 is functionally important for the expression of dopamine-mediated behaviors and that the molecular mechanisms involved in the β-arrestin2-dependent Akt/GSK3 (glycogen synthase kinase type 3) signaling cascade may provide novel potential pharmacological targets with which to manage dopamine-related neurological and psychiatric disorders (127, 129).

SUMMARY

GRK and Arrestin Functions

Studies in several systems described above demonstrate that GRKs and arrestins do regulate GPCRs in vivo and that the failure to regulate GPCRs properly can have profound physiological consequences. In the absence of elements of the GRK-arrestin desensitization machinery, GPCRs can remain abnormally supersensitive to agonist stimulation. This manifests as prolonged and exaggerated responses to agonist challenges. In the clearest examples, a receptor subtype appears to require only one particular GRK or arrestin protein to be regulated properly. The D2-like dopamine receptors in striatal medium spiny neurons fail to desensitize in the absence of GRK6, whereas brain M2 muscarinic receptors mediating several responses fail to desensitize in the absence of GRK5. Similarly, β-arrestin2 is required for μ-opioid receptor desensitization, whereas β-arrestin1 appears ineffective at regulating this receptor. Thus, the role of GRKs and arrestins defined in model cell systems and using purified proteins can be validated in the physiological responses of living animals.

In contrast, GRK and arrestin knockout mice are grossly normal (with the one exception of the embryonically lethal GRK2 knockout), which is consistent with the role of

the GRK-arrestin system to regulate primarily agonist-activated receptors. In the absence of the stress of a high level of receptor stimulation, the relative inability of these animals to dampen excessive signaling is not immediately evident.

Although GRKs and arrestins have been shown to promote G protein–independent signaling pathways through arrestin-scaffolded signaling proteins in model cell systems, evidence for such pathways in vivo is scant at present. The identification of β-arrestin2 scaffolding–dependent regulation of Akt in the striatum is the clearest example of such function to date. Increased interest in this type of signaling in recent years is sure to focus needed attention on identifying physiological systems in which this role of the GRK-arrestin system can be clearly demonstrated.

GRK and Arrestin Specificity

Because there are several hundred GPCRs but only four widely distributed GRKs and two widely distributed β-arrestin proteins to regulate them, there remains the issue of defining the wiring diagram with which GRKs and arrestins regulate any particular GPCR. Current evidence suggests that this may be quite complex indeed.

Some receptors appear to be regulated by one particular GRK and/or arrestin protein, as with the D2-like receptors in striatum by GRK6 or the μ-opioid receptor by β-arrestin2. In contrast, other receptors do not exhibit a dramatic alteration in signaling with the loss of any one GRK or β-arrestin, suggesting that many GRK subtypes or both β-arrestins all contribute to regulation and can compensate for the loss of any one of their number. The only exception is mice lacking GRK2 (19) or both β-arrestin1 and β-arrestin2 (9); such phenotypes are embryonically lethal. The most curious and informative finding is that a single GPCR subtype may be regulated by distinct GRKs when it is present in different cell types. In the CNS and lung, M2 muscarinic receptors appear to be regulated primarily by GRK5. However, despite the prominent expression of both M2 receptors and GRK5 in the heart, cardiac M2 muscarinic receptors seem regulated quite normally in GRK5 knockout mice. Thus, to understand the regulation of a specific receptor, it is critical to examine that receptor in its native context. Information garnered from model cell systems, or even from examining other organs of an intact animal, may not be informative for the same receptor subtype in a different environment. Given the few receptors and physiological responses examined in detail to date, it remains unclear whether most receptors are regulated by exclusive GRK-arrestin pathways or promiscuously by multiple GRKs and/or both β-arrestins.

Receptors on distinct cell types can also respond differently to loss of a particular GRK or arrestin. The SDF-1-activated CXCR4 receptor appears to be regulated by GRK6 in both T lymphocytes and neutrophils, but loss of GRK6 has opposite effects on chemotaxis in these two cell types: T cells lacking GRK6 chemotax less well toward SDF-1, whereas neutrophils chemotax markedly better toward SDF-1. The basis for this difference remains obscure but may reflect differences in the regulation of downstream pathways leading to chemotaxis in these different cells.

The physiological significance of GRK-arrestin regulation of GPCRs that the studies described here have demonstrated strongly suggests that future development of inhibitors or modulators of these regulatory proteins and their processes is an effective approach by which to fine-tune physiological functions. For example, the ability of some tastants to induce lingering aftertaste may depend on their ability to inhibit GRKs in taste cells that putatively regulate tastant receptors (58a). This and other examples described herein demonstrate the feasibility of pharmacological approaches of modulating GPCR regulation to correct abnormal physiological function in disease.

The lessons learned so far from these in vivo studies only highlight the many further studies that remain to be undertaken and the myriad details of which we remain ignorant. Physiological regulation of nearly all hormone and neurotransmitter receptors by GRK-arrestin pathways remains untested. The widespread regulation of GRK expression levels in various physiological and pathopysiological conditions adds another level of complexity. These GRK dynamics, coupled with examples of substantial physiological changes in GRK6-heterozygous knockout mice in several systems, suggest that GRK-arrestin regulation of a GPCR can differ greatly even in a single organ, depending on the conditions that regulate GRK expression. Furthermore, the finding that GRK4 polymorphisms in humans can alter kidney dopamine receptor responses under apparently basal conditions adds the potential for yet more complexity. Thus, we have a long way to go to define in detail how particular receptors are regulated by these GRK-arrestin pathways.

SUMMARY POINTS

1. The failure to regulate GPCRs properly can have profound physiological consequences.
2. In the absence of elements of the GRK-arrestin desensitization machinery, GPCRs can exhibit prolonged and exaggerated responses to agonist challenge owing to disrupted desensitization.
3. Deficiency in GRK-arrestin mechanisms may also cause a decrease in responses owing to disrupted G protein–independent, arrestin-mediated signaling.
4. Some receptors appear to be regulated by one particular GRK and/or arrestin protein, whereas other receptors appear to be regulated by many GRK subtypes or both β-arrestins.
5. A single GPCR subtype may be regulated by distinct GRKs when it is present in different cell types.
6. Receptors on distinct cell types can also respond differently to loss of a particular GRK or arrestin.
7. The widespread regulation of GRK expression levels in various physiological and pathophysiological conditions suggests that GRK-arrestin regulation of a GPCR can differ greatly even in a single organ, depending on the conditions that regulate GRK expression.

FUTURE ISSUES

1. The complex wiring diagram with which multiple GRKs and arrestins regulate any particular GPCR in a specific cell group needs to be defined.
2. Physiological functions of individual GRKs and arrestins in specific responses in living organisms should be identified.
3. The physiological role of processes governing GPCR desensitization in a specific function requires further understanding.

4. The recent discovery of G protein–independent, arrestin-mediated signaling of GPCRs should spur efforts to identify physiological systems in which this role of the GRK-arrestin system is predominant.
5. GPCR-desensitization-related versus G protein–independent signaling–related functions of GPCR regulation by individual GRKs and arrestins, should be delineated.

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LITERATURE CITED

1. Pierce KL, Premont RT, Lefkowitz RJ. 2002. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3:639–50
2. Luttrell LM. 2005. Composition and function of G protein-coupled receptor signalsomes controlling mitogen-activated protein kinase activity. *J. Mol. Neurosci.* 26:253–64
3. Lefkowitz RJ, Shenoy SK. 2005. Transduction of receptor signals by β-arrestins. *Science* 308:512–17
4. Hausdorff WP, Caron MG, Lefkowitz RJ. 1990. Turning off the signal: desensitization of β-adrenergic receptor function. *FASEB J.* 4:2881–89
5. Premont RT. 2005. Once and future signaling: G protein-coupled receptor kinase control of neuronal sensitivity. *Neuromol. Med.* 7:129–47
6. Penn RB, Pronin AN, Benovic JL. 2000. Regulation of G protein-coupled receptor kinases. *Trends Cardiovasc. Med.* 10:81–89
7. Ferguson SS. 2001. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol. Rev.* 53:1–24
8. Penela P, Ribas C, Mayor FJ. 2003. Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. *Cell Signal* 15:973–81
9. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. 2004. Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.* 27:107–44
10. Gurevich VV, Gurevich EV. 2004. The molecular acrobatics of arrestin activation. *Trends Pharmacol. Sci.* 25:105–11
11. Gainetdinov RR, Premont RT, Caron MG, Lefkowitz RJ. 2000. Reply: receptor specificity of G-protein-coupled receptor kinases. *Trends Pharmacol. Sci.* 21:366–7
12. Chen CK. 2002. Recoverin and rhodopsin kinase. *Adv. Exp. Med. Biol.* 514:101–7
13. Arshavsky VY. 2002. Rhodopsin phosphorylation: from terminating single photon responses to photoreceptor dark adaptation. *Trends Neurosci.* 25:124–6
14. Maeda T, Imanishi Y, Palczewski K. 2003. Rhodopsin phosphorylation: 30 years later. *Prog. Retin. Eye Res.* 22:417–34
15. Koch WJ. 2004. Genetic and phenotypic targeting of β-adrenergic signaling in heart failure. *Mol. Cell Biochem.* 263:5–9
16. Hata JA, Williams ML, Koch WJ. 2004. Genetic manipulation of myocardial β-adrenergic receptor activation and desensitization. *J. Mol. Cell Cardiol.* 37:11–21
17. Iaccarino G, Koch WJ. 2003. Transgenic mice targeting the heart unveil G protein-coupled receptor kinases as therapeutic targets. *Assay Drug Dev. Technol.* 1:347–55

18. Rockman HA, Choi DJ, Akhter SA, Jaber M, Giros B, et al. 1998. Control of myocardial contractile function by the level of β-adrenergic receptor kinase 1 in gene-targeted mice. *J. Biol. Chem.* 273:18180–84
19. Jaber M, Koch WJ, Rockman H, Smith B, Bond RA, et al. 1996. Essential role of β-adrenergic receptor kinase 1 in cardiac development and function. *Proc. Natl. Acad. Sci. USA* 93:12974–79
20. Walker JK, Peppel K, Lefkowitz RJ, Caron MG, Fisher JT. 1999. Altered airway and cardiac responses in mice lacking G protein-coupled receptor kinase 3. *Am. J. Physiol.* 276:R1214–21
21. Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, et al. 1997. β-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to β-adrenergic stimulation. *Circ. Res.* 81:1021–26
22. Gros R, Tan CM, Chorazyczewski J, Kelvin DJ, Benovic JL, Feldman RD. 1999. G-protein-coupled receptor kinase expression in hypertension. *Clin. Pharmacol. Ther.* 65:545–51
23. Gros R, Benovic JL, Tan CM, Feldman RD. 1997. G-protein-coupled receptor kinase activity is increased in hypertension. *J. Clin. Invest.* 99:2087–93
24. Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS, Feldman RD. 2000. G-Protein-coupled receptor kinase activity in hypertension: increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* 35:38–42
25. Eckhart AD, Ozaki T, Tevaearai H, Rockman HA, Koch WJ. 2002. Vascular-targeted overexpression of G protein-coupled receptor kinase-2 in transgenic mice attenuates β-adrenergic receptor signaling and increases resting blood pressure. *Mol. Pharmacol.* 61:749–58
26. Keys JR, Zhou RH, Harris DM, Druckman CA, Eckhart AD. 2005. Vascular smooth muscle overexpression of G protein-coupled receptor kinase 5 elevates blood pressure, which segregates with sex and is dependent on G_i-mediated signaling. *Circulation* 112:1145–53
27. Zeng C, Sanada H, Watanabe H, Eisner GM, Felder RA, Jose PA. 2004. Functional genomics of the dopaminergic system in hypertension. *Physiol. Genom.* 19:233–46
28. Jose PA, Eisner GM, Felder RA. 1998. Renal dopamine receptors in health and hypertension. *Pharmacol. Ther.* 80:149–82
29. Felder RA, Sanada H, Xu J, Yu PY, Wang Z, et al. 2002. G protein-coupled receptor kinase 4 gene variants in human essential hypertension. *Proc. Natl. Acad. Sci. USA* 99:3872–77
30. Rankin ML, Marinec PS, Cabrera DM, Wang Z, Jose PA, Sibley DR. 2006. The D1 dopamine receptor is constitutively phosphorylated by G protein-coupled receptor kinase 4. *Mol. Pharmacol.* 69:759–69
31. Sanada H, Yatabe J, Midorikawa S, Katoh T, Hashimoto S, et al. 2006. Amelioration of genetic hypertension by suppression of renal G protein-coupled receptor kinase type 4 expression. *Hypertension* 47:1131–39
32. Sanada H, Yatabe J, Midorikawa S, Hashimoto S, Watanabe T, et al. 2006. Single-nucleotide polymorphisms for diagnosis of salt-sensitive hypertension. *Clin. Chem.* 52:352–60
33. Lohmueller KE, Wong LJ, Mauney MM, Jiang L, Felder RA, et al. 2006. Patterns of genetic variation in the hypertension candidate gene GRK4: ethnic variation and haplotype structure. *Ann. Hum. Genet.* 70:27–41
34. Gu D, Su S, Ge D, Chen S, Huang J, et al. 2006. Association study with 33 single-nucleotide polymorphisms in 11 candidate genes for hypertension in Chinese. *Hypertension* 47:1147–54

29. Identifies GRK4 gene variants in human essential hypertension.

35. Liu S, Premont RT, Kontos CD, Zhu S, Rockey DC. 2005. A crucial role for GRK2 in regulation of endothelial cell nitric oxide synthase function in portal hypertension. *Nat. Med.* 11:952–58
36. Liu S, Premont RT, Kontos CD, Huang J, Rockey DC. 2003. Endothelin-1 activates endothelial cell nitric-oxide synthase via heterotrimeric G-protein βγ subunit signaling to protein kinase B/Akt. *J. Biol. Chem.* 278:49929–35
37. De Blasi A, Parruti G, Sallese M. 1995. Regulation of G protein-coupled receptor kinase subtypes in activated T lymphocytes. Selective increase of β-adrenergic receptor kinase 1 and 2. *J. Clin. Invest.* 95:203–10
38. Loudon RP, Perussia B, Benovic JL. 1996. Differentially regulated expression of the G-protein-coupled receptor kinases, βARK and GRK6, during myelomonocytic cell development in vitro. *Blood* 88:4547–57
39. Lombardi MS, Kavelaars A, Schedlowski M, Bijlsma JW, Okihara KL, et al. 1999. Decreased expression and activity of G-protein-coupled receptor kinases in peripheral blood mononuclear cells of patients with rheumatoid arthritis. *FASEB J.* 13:715–25
40. Lombardi MS, Kavelaars A, Penela P, Scholtens EJ, Roccio M, et al. 2002. Oxidative stress decreases G protein-coupled receptor kinase 2 in lymphocytes via a calpain-dependent mechanism. *Mol. Pharmacol.* 62:379–88
41. Lombardi MS, Kavelaars A, Cobelens PM, Schmidt RE, Schedlowski M, Heijnen CJ. 2001. Adjuvant arthritis induces down-regulation of G protein-coupled receptor kinases in the immune system. *J. Immunol.* 166:1635–40
42. Vroon A, Lombardi MS, Kavelaars A, Heijnen CJ. 2003. Changes in the G-protein-coupled receptor desensitization machinery during relapsing-progressive experimental allergic encephalomyelitis. *J. Neuroimmunol.* 137:79–86
43. Oyama N, Urasawa K, Kaneta S, Sakai H, Saito T, et al. 2005. Chronic beta-adrenergic receptor stimulation enhances the expression of G-protein coupled receptor kinases, GRK2 and GRK5, in both the heart and peripheral lymphocytes. *Circ. J.* 69:987–90
44. Fong AM, Premont RT, Richardson RM, Yu YR, Lefkowitz RJ, Patel DD. 2002. Defective lymphocyte chemotaxis in β-arrestin2- and GRK6-deficient mice. *Proc. Natl. Acad. Sci. USA* 99:7478–83
45. Kavelaars A, Vroon A, Raatgever RP, Fong AM, Premont RT, et al. 2003. Increased acute inflammation, leukotriene B4-induced chemotaxis, and signaling in mice deficient for G protein-coupled receptor kinase 6. *J. Immunol.* 171:6128–34
46. Vroon A, Heijnen CJ, Raatgever R, Touw IP, Ploemacher RE, et al. 2004. GRK6 deficiency is associated with enhanced CXCR4-mediated neutrophil chemotaxis in vitro and impaired responsiveness to G-CSF in vivo. *J. Leukoc. Biol.* 75:698–704
47. Vroon A, Heijnen CJ, Lombardi MS, Cobelens PM, Mayor FJ, et al. 2004. Reduced GRK2 level in T cells potentiates chemotaxis and signaling in response to CCL4. *J. Leukoc. Biol.* 75:901–9
48. Vroon A, Kavelaars A, Limmroth V, Lombardi MS, Goebel MU, et al. 2005. G protein-coupled receptor kinase 2 in multiple sclerosis and experimental autoimmune encephalomyelitis. *J. Immunol.* 174:4400–6
49. Wang Y, Tang Y, Teng L, Wu Y, Zhao X, Pei G. 2006. Association of β-arrestin and TRAF6 negatively regulates Toll-like receptor-interleukin 1 receptor signaling. *Nat. Immunol.* 7:139–47
50. Su Y, Raghuwanshi SK, Yu Y, Nanney LB, Richardson RM, Richmond A. 2005. Altered CXCR2 signaling in β-arrestin-2-deficient mouse models. *J. Immunol.* 175:5396–402

44. Demonstrates the role of β-arrestin2 and GRK6 in lymphocyte chemotaxis.

45. Shows the role of GRK6 in neutrophil chemotaxis and inflammation.

51. Describes M2 muscarinic regulation by GRK5 in the airway, but not in the heart.

52. Uncovers the role of β-arrestin-2 in the development of allergic asthma.

51. Walker JK, Gainetdinov RR, Feldman DS, McFawn PK, Caron MG, et al. 2004. G protein-coupled receptor kinase 5 regulates airway responses induced by muscarinic receptor activation. *Am. J. Physiol. Lung Cell Mol. Physiol.* 286:L312–19

52. Walker JK, Fong AM, Lawson BL, Savov JD, Patel DD, et al. 2003. β-Arrestin-2 regulates the development of allergic asthma. *J. Clin. Invest.* 112:566–74

53. Bliziotes M, Murtagh J, Wiren K. 1996. β-adrenergic receptor kinase-like activity and β-arrestin are expressed in osteoblastic cells. *J. Bone Miner. Res.* 11:820–26

54. Spurney RF, Flannery PJ, Garner SC, Athirakul K, Liu S, et al. 2002. Anabolic effects of a G protein-coupled receptor kinase inhibitor expressed in osteoblasts. *J. Clin. Invest.* 109:1361–71

55. Wang L, Quarles LD, Spurney RF. 2004. Unmasking the osteoinductive effects of a G-protein-coupled receptor (GPCR) kinase (GRK) inhibitor by treatment with PTH(1–34). *J. Bone Miner. Res.* 19:1661–70

56. Bouxsein ML, Pierroz DD, Glatt V, Goddard DS, Cavat F, et al. 2005. β-Arrestin2 regulates the differential response of cortical and trabecular bone to intermittent PTH in female mice. *J. Bone Miner. Res.* 20:635–43

57. Ferrari SL, Pierroz DD, Glatt V, Goddard DS, Bianchi EN, et al. 2005. Bone response to intermittent parathyroid hormone is altered in mice null for β-arrestin2. *Endocrinology* 146:1854–62

58. Vassilatis DK, Hohmann JG, Zeng H, Li F, Ranchalis JE, et al. 2003. The G protein-coupled receptor repertoires of human and mouse. *Proc. Natl. Acad. Sci. USA* 100:4903–8

58a. Zubare-Samuelov M, Shaul ME, Peri I, Aliluiko A, Tirosh O, Naim M. 2005. Inhibition of signal termination-related kinases by membrane-permeant bitter and sweet tastants: potential role in taste signal termination. *Am. J. Physiol. Cell Physiol.* 289:C483–92

59. Arriza JL, Dawson TM, Simerly RB, Martin LJ, Caron MG, et al. 1992. The G-protein-coupled receptor kinases βARK1 and βARK2 are widely distributed at synapses in rat brain. *J. Neurosci.* 12:4045–55

60. Erdtmann-Vourliotis M, Mayer P, Ammon S, Riechert U, Hollt V. 2001. Distribution of G-protein-coupled receptor kinase (GRK) isoforms 2, 3, 5 and 6 mRNA in the rat brain. *Br. Res. Mol. Br. Res.* 95:129–37

61. Penela P, Alvarez-Dolado M, Munoz A, Mayor FJ. 2000. Expression patterns of the regulatory proteins G protein-coupled receptor kinase 2 and β-arrestin 1 during rat postnatal brain development: effect of hypothyroidism. *Eur. J. Biochem.* 267:4390–96

62. Grange-Midroit M, Garcia-Sevilla JA, Ferrer-Alcon M, La Harpe R, Huguelet P, Guimon J. 2003. Regulation of GRK 2 and 6, β-arrestin-2 and associated proteins in the prefrontal cortex of drug-free and antidepressant drug-treated subjects with major depression. *Br. Res. Mol. Br. Res.* 111:31–41

63. Koh PO, Undie AS, Kabbani N, Levenson R, Goldman-Rakic PS, Lidow MS. 2003. Up-regulation of neuronal calcium sensor-1 (NCS-1) in the prefrontal cortex of schizophrenic and bipolar patients. *Proc. Natl. Acad. Sci. USA* 100:313–17

64. Kabbani N, Negyessy L, Lin R, Goldman-Rakic P, Levenson R. 2002. Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor. *J. Neurosci.* 22:8476–86

65. Bezard E, Gross CE, Qin L, Gurevich VV, Benovic JL, Gurevich EV. 2005. L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain. *Neurobiol. Dis.* 18:323–35

66. Terwilliger RZ, Ortiz J, Guitart X, Nestler EJ. 1994. Chronic morphine administration increases β-adrenergic receptor kinase (βARK) levels in the rat locus coeruleus. *J. Neurochem.* 63:1983–86

67. Ozaita A, Escriba PV, Ventayol P, Murga C, Mayor FJ, Garcia-Sevilla JA. 1998. Regulation of G protein-coupled receptor kinase 2 in brains of opiate-treated rats and human opiate addicts. *J. Neurochem*. 70:1249–57
68. Hurle MA. 2001. Changes in the expression of G protein-coupled receptor kinases and β-arrestin 2 in rat brain during opioid tolerance and supersensitivity. *J. Neurochem*. 77:486–92
69. Diaz A, Pazos A, Florez J, Ayesta FJ, Santana V, Hurle MA. 2002. Regulation of μ-opioid receptors, G-protein-coupled receptor kinases and β-arrestin 2 in the rat brain after chronic opioid receptor antagonism. *Neuroscience* 112:345–53
70. Narita M, Suzuki M, Narita M, Niikura K, Nakamura A, et al. 2006. μ-Opioid receptor internalization-dependent and -independent mechanisms of the development of tolerance to μ-opioid receptor agonists: comparison between etorphine and morphine. *Neuroscience* 138:609–19
71. Li AH, Wang HL. 2001. G protein-coupled receptor kinase 2 mediates μ-opioid receptor desensitization in GABAergic neurons of the nucleus raphe magnus. *J. Neurochem*. 77:435–44
72. Fan X, Zhang J, Zhang X, Yue W, Ma L. 2002. Acute and chronic morphine treatments and morphine withdrawal differentially regulate GRK2 and GRK5 gene expression in rat brain. *Neuropharmacology* 43:809–16
73. Ammon-Treiber S, Hollt V. 2005. Morphine-induced changes of gene expression in the brain. *Addict. Biol*. 10:81–89
74. Bohn LM, Gainetdinov RR, Caron MG. 2004. G protein-coupled receptor kinase/beta-arrestin systems and drugs of abuse: psychostimulant and opiate studies in knockout mice. *Neuromol. Med*. 5:41–50
75. Pronin AN, Morris AJ, Surguchov A, Benovic JL. 2000. Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *J. Biol. Chem*. 275:26515–22
76. Suo Z, Wu M, Citron BA, Wong GT, Festoff BW. 2004. Abnormality of G-protein-coupled receptor kinases at prodromal and early stages of Alzheimer's disease: an association with early β-amyloid accumulation. *J. Neurosci*. 24:3444–52
77. Lombardi MS, van den Tweel E, Kavelaars A, Groenendaal F, van Bel F, Heijnen CJ. 2004. Hypoxia/ischemia modulates G protein-coupled receptor kinase 2 and β-arrestin-1 levels in the neonatal rat brain. *Stroke* 35:981–86
78. Willets JM, Nahorski SR, Challiss RA. 2005. Roles of phosphorylation-dependent and -independent mechanisms in the regulation of M1 muscarinic acetylcholine receptors by G protein-coupled receptor kinase 2 in hippocampal neurons. *J. Biol. Chem*. 280:18950–58
79. Kageyama K, Hanada K, Moriyama T, Nigawara T, Sakihara S, Suda T. 2006. G protein-coupled receptor kinase 2 involvement in desensitization of corticotropin-releasing factor (CRF) receptor type 1 by CRF in murine corticotrophs. *Endocrinology* 147:441–50
80. Boekhoff I, Inglese J, Schleicher S, Koch WJ, Lefkowitz RJ, Breer H. 1994. Olfactory desensitization requires membrane targeting of receptor kinase mediated by βγ-subunits of heterotrimeric G proteins. *J. Biol. Chem*. 269:37–40
81. Diverse-Pierluissi M, Inglese J, Stoffel RH, Lefkowitz RJ, Dunlap K. 1996. G protein-coupled receptor kinase mediates desensitization of norepinephrine-induced Ca^{2+} channel inhibition. *Neuron* 16:579–85
82. Peppel K, Boekhoff I, McDonald P, Breer H, Caron MG, Lefkowitz RJ. 1997. G protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of odorant receptor desensitization. *J. Biol. Chem*. 272:25425–28

83. Niculescu AB, Segal DS, Kuczenski R, Barrett T, Hauger RL, Kelsoe JR. 2000. Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. *Physiol. Genom*. 4:83–91

84. Barrett TB, Hauger RL, Kennedy JL, Sadovnick AD, Remick RA, et al. 2003. Evidence that a single nucleotide polymorphism in the promoter of the G protein receptor kinase 3 gene is associated with bipolar disorder. *Mol. Psychiat.* 8:546–57

84. Presents evidence for a role of GRK3 in bipolar disorder.

85. Shaltiel G, Shamir A, Levi I, Bersudsky Y, Agam G. 2006. Lymphocyte G-protein receptor kinase (GRK)3 mRNA levels in bipolar disorder. *Int. J. Neuropsychopharmacol.* 9:761–66 doi: 10.1017/S146114570500636X

86. Yu SY, Takahashi S, Arinami T, Ohkubo T, Nemoto Y, et al. 2004. Mutation screening and association study of the β-adrenergic receptor kinase 2 gene in schizophrenia families. *Psychiat. Res.* 125:95–104

87. Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. 1999. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283:397–401

88. Kim KM, Gainetdinov RR, Laporte SA, Caron MG, Barak LS. 2005. G protein-coupled receptor kinase regulates dopamine D3 receptor signaling by modulating the stability of a receptor-filamin-β-arrestin complex. A case of autoreceptor regulation. *J. Biol. Chem*. 280:12774–80

89. Terman GW, Jin W, Cheong YP, Lowe J, Caron MG, et al. 2004. G-protein receptor kinase 3 (GRK3) influences opioid analgesic tolerance but not opioid withdrawal. *Br. J. Pharmacol.* 141:55–64

90. Xu M, Petraschka M, McLaughlin JP, Westenbroek RE, Caron MG, et al. 2004. Neuropathic pain activates the endogenous κ opioid system in mouse spinal cord and induces opioid receptor tolerance. *J. Neurosci.* 24:4576–84

91. McLaughlin JP, Myers LC, Zarek PE, Caron MG, Lefkowitz RJ, et al. 2004. Prolonged κ opioid receptor phosphorylation mediated by G-protein receptor kinase underlies sustained analgesic tolerance. *J. Biol. Chem.* 279:1810–18

92. Sallese M, Salvatore L, D'Urbano E, Sala G, Storto M, et al. 2000. The G-protein-coupled receptor kinase GRK4 mediates homologous desensitization of metabotropic glutamate receptor 1. *FASEB J.* 14:2569–80

93. Iacovelli L, Salvatore L, Capobianco L, Picascia A, Barletta E, et al. 2003. Role of G protein-coupled receptor kinase 4 and β-arrestin 1 in agonist-stimulated metabotropic glutamate receptor 1 internalization and activation of mitogen-activated protein kinases. *J. Biol. Chem*. 278:12433–42

94. Perroy J, Adam L, Qanbar R, Chenier S, Bouvier M. 2003. Phosphorylation-independent desensitization of GABA(B) receptor by GRK4. *EMBO J*. 22:3816–24

95. Gurevich EV, Benovic JL, Gurevich VV. 2004. Arrestin2 expression selectively increases during neural differentiation. *J. Neurochem*. 91:1404–16

96. Gainetdinov RR, Bohn LM, Walker JK, Laporte SA, Macrae AD, et al. 1999. Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. *Neuron* 24:1029–36

96. Describes pronounced behavioral and biochemical muscarinic supersensitivity in GRK5-deficient mice.

97. Gomeza J, Shannon H, Kostenis E, Felder C, Zhang L, et al. 1999. Pronounced pharmacologic deficits in M2 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. USA* 96:1692–97

98. Fehr C, Fickova M, Hiemke C, Reuss S, Dahmen N. 1997. Molecular cloning of rat G-protein-coupled receptor kinase 6 (GRK6) from brain tissue, and its mRNA expression in different brain regions and peripheral tissues. *Br. Res. Mol. Br. Res*. 49:278–82

99. Gainetdinov RR, Bohn LM, Sotnikova TD, Cyr M, Laakso A, et al. 2003. Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. *Neuron* 38:291–303

100. Sotnikova TD, Budygin EA, Jones SR, Dykstra LA, Caron MG, Gainetdinov RR. 2004. Dopamine transporter-dependent and -independent actions of trace amine β-phenylethylamine. *J. Neurochem*. 91:362–73

101. Premont RT, Gainetdinov RR, Caron MG. 2001. Following the trace of elusive amines. *Proc. Natl. Acad. Sci. USA* 98:9474–75

102. Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, et al. 2005. Dopamine supersensitivity correlates with D2 high states, implying many paths to psychosis. *Proc. Natl. Acad. Sci. USA* 102:3513–18

103. Ferrer-Alcon M, La Harpe R, Garcia-Sevilla JA. 2004. Decreased immunodensities of μ-opioid receptors, receptor kinases GRK 2/6 and β-arrestin-2 in postmortem brains of opiate addicts. *Br. Res. Mol. Br. Res*. 121:114–22

104. Attramadal H, Arriza JL, Aoki C, Dawson TM, Codina J, et al. 1992. β-Arrestin2, a novel member of the arrestin/β-arrestin gene family. *J. Biol. Chem*. 267:17882–90

105. Parruti G, Ambrosini G, Sallese M, De Blasi A. 1993. Molecular cloning, functional expression and mRNA analysis of human β-adrenergic receptor kinase 2. *Biochem. Biophys. Res. Commun*. 190:475–81

106. Gurevich EV, Benovic JL, Gurevich VV. 2002. Arrestin2 and arrestin3 are differentially expressed in the rat brain during postnatal development. *Neuroscience* 109:421–36

107. Kittel A, Komori N. 1999. Ultrastructural localization of β-arrestin-1 and -2 in rat lumbar spinal cord. *J. Comp. Neurol*. 412:649–55

108. Fan XL, Zhang JS, Zhang XQ, Yue W, Ma L. 2003. Differential regulation of β-arrestin 1 and β-arrestin 2 gene expression in rat brain by morphine. *Neuroscience* 117:383–89

109. Avissar S, Matuzany-Ruban A, Tzukert K, Schreiber G. 2004. β-Arrestin-1 levels: reduced in leukocytes of patients with depression and elevated by antidepressants in rat brain. *Am. J. Psychiat*. 161:2066–72

110. Ohsawa M, Mizoguchi H, Narita M, Nagase H, Dun NJ, Tseng LF. 2003. Involvement of β-arrestin-2 in modulation of the spinal antinociception induced by μ-opioid receptor agonists in the mouse. *Neurosci. Lett*. 346:13–16

111. Zhang J, Ferguson SS, Barak LS, Bodduluri SR, Laporte SA, et al. 1998. Role for G protein-coupled receptor kinase in agonist-specific regulation of μ-opioid receptor responsiveness. *Proc. Natl. Acad. Sci. USA* 95:7157–62

112. Whistler JL, von Zastrow M. 1998. Morphine-activated opioid receptors elude desensitization by β-arrestin. *Proc. Natl. Acad. Sci. USA* 95:9914–19

113. Kovoor A, Celver JP, Wu A, Chavkin C. 1998. Agonist induced homologous desensitization of μ-opioid receptors mediated by G protein-coupled receptor kinases is dependent on agonist efficacy. *Mol. Pharmacol*. 54:704–11

114. Haberstock-Debic H, Kim KA, Yu YJ, von Zastrow M. 2005. Morphine promotes rapid, arrestin-dependent endocytosis of μ-opioid receptors in striatal neurons. *J. Neurosci*. 25:7847–57

115. Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. 1999. Enhanced morphine analgesia in mice lacking β-arrestin 2. *Science* 286:2495–98

116. Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ, Caron MG. 2000. μ-opioid receptor desensitization by β-arrestin-2 determines morphine tolerance but not dependence. *Nature* 408:720–23

99. Reports disruption of dopamine receptor desensitization and behavioral abnormalities in GRK6-deficient mice.

115. Found abnormal μ-opioid receptor regulation and enhanced morphine analgesia in mice lacking β-arrestin 2.

117. Beaulieu JM. 2005. Morphine-induced μ-opioid receptor internalization: a paradox solved in neurons. *J. Neurosci*. 25:10061–63
118. Bohn LM, Lefkowitz RJ, Caron MG. 2002. Differential mechanisms of morphine antinociceptive tolerance revealed in βarrestin-2 knock-out mice. *J. Neurosci*. 22:10494–500
119. Bohn LM, Dykstra LA, Lefkowitz RJ, Caron MG, Barak LS. 2004. Relative opioid efficacy is determined by the complements of the G protein-coupled receptor desensitization machinery. *Mol. Pharmacol*. 66:106–12
120. Raehal KM, Walker JK, Bohn LM. 2005. Morphine side effects in β-arrestin 2 knockout mice. *J. Pharmacol. Exp. Ther*. 314:1195–201
121. Jiang B, Shi Y, Li H, Kang L, Ma L. 2006. Decreased morphine analgesia in rat overexpressing β-arrestin 2 at periaqueductal gray. *Neurosci. Lett*. 400:150–53
122. Hack SP, Bagley EE, Chieng BC, Christie MJ. 2005. Induction of δ-opioid receptor function in the midbrain after chronic morphine treatment. *J. Neurosci*. 25:3192–98
123. Bradaia A, Berton F, Ferrari S, Luscher C. 2005. β-Arrestin2, interacting with phosphodiesterase 4, regulates synaptic release probability and presynaptic inhibition by opioids. *Proc. Natl. Acad. Sci. USA* 102:3034–39
124. Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, Wise RA. 2002. Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. *J. Neurosci.* 22:RC224
125. Bohn LM, Gainetdinov RR, Sotnikova TD, Medvedev IO, Lefkowitz RJ, et al. 2003. Enhanced rewarding properties of morphine, but not cocaine, in βarrestin-2 knock-out mice. *J. Neurosci.* 23:10265–73
126. Tao R, Auerbach SB. 1995. Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. *Neuroscience* 68:553–61
127. Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. 2005. An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 122:261–73
128. Gainetdinov RR, Caron MG. 2003. Monoamine transporters: from genes to behavior. *Annu. Rev. Pharmacol. Toxicol*. 43:261–84
129. Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, et al. 2004. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. USA* 101:5099–104

127. Identified role of G protein–independent, β-arrestin 2–mediated signaling in dopaminergic neurotransmission and behavior.

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