

DESENSITIZATION OF G PROTEIN–COUPLED RECEPTORS AND NEURONAL FUNCTIONS

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■ **Abstract** G protein–coupled receptors (GPCRs) have proven to be the most highly favorable class of drug targets in modern pharmacology. Over 90% of nonsensory GPCRs are expressed in the brain, where they play important roles in numerous neuronal functions. GPCRs can be desensitized following activation by agonists by becoming phosphorylated by members of the family of G protein–coupled receptor kinases (GRKs). Phosphorylated receptors are then bound by arrestins, which prevent further stimulation of G proteins and downstream signaling pathways. Discussed in this review are recent progress in understanding basics of GPCR desensitization, novel functional roles, patterns of brain expression, and receptor specificity of GRKs and β arrestins in major brain functions. In particular, screening of genetically modified mice lacking individual GRKs or β arrestins for alterations in behavioral and biochemical responses to cocaine and morphine has revealed a functional specificity in dopamine and μ -opioid receptor regulation of locomotion and analgesia. An important and specific role of GRKs and β arrestins in regulating physiological responsiveness to psychostimulants and morphine suggests potential involvement of these molecules in certain brain disorders, such as addiction, Parkinson's disease, mood disorders, and schizophrenia. Furthermore, the utility of a pharmacological strategy aimed at targeting this GPCR desensitization machinery to regulate brain functions can be envisaged.

INTRODUCTION

The cell-surface receptors for most neuromodulators are members of the large superfamily of G protein–coupled receptors (GPCRs). These receptors share similar primary amino acid sequences, a common seven–transmembrane–spanning domain architecture, and the ability to modulate intracellular metabolism through

the activation of heterotrimeric GTP-binding proteins (G proteins) (Hamm & Gilchrist 1996, Watson & Arkininstall 1994). GPCRs exist for many biologically active molecules such as amines (dopamine, noradrenaline, serotonin, histamine), amino acid transmitters (glutamate, GABA), peptides (opioids, tachykinins, neurotensin, somatostatin, cholecystokinin, gut-brain peptides such as GLP-1 and VIP, and most endocrine-releasing factors), and lipid-derived products (lysophosphatidic acid, sphingosine-1-phosphate, eicosinoids). GPCRs thus mediate a large variety of physiological events throughout the body, from chemosensory recognition (vision, olfaction, taste) to endocrine regulation to complex behavioral events. Indeed, over 360 nonsensory GPCRs, which are activated by about 200 endogenous substances, have been characterized, and over 160 orphan GPCRs remain whose natural ligands are still unknown (Wise et al. 2004). Of these nonsensory GPCRs, over 90% are expressed in the brain (Vassilatis et al. 2003).

In the CNS, GPCRs function primarily, but not exclusively, as mediators of slow neuromodulators rather than fast neurotransmitters, and their role is critical to normal brain function. Under- or overactivity of many individual GPCR systems in the brain may contribute to pathological conditions, ranging from hypodopaminergic movement disorders to mania and depression. Thus these receptors are primary or downstream targets for a variety of useful therapeutic agents and continue to be the focus of intense pharmaceutical development (Wise et al. 2004).

GPCR Signaling

In the absence of the appropriate activating ligand or agonist, both receptors and G proteins are generally inactive. GPCRs respond to the presence of their activating ligands or agonists by activating coupled G proteins. Over a range of agonist concentrations, G protein activation is proportionate with receptor binding by the activating ligand.

Each receptor subtype can couple to and activate only certain G protein types, each leading to distinct downstream signals. G proteins consist of three associated protein subunits, called α , β , and γ (Hamm & Gilchrist 1996, Watson & Arkininstall 1994). G proteins are classified based on their α -subunits, and there are 15 known α -subunits that have been categorized into four subfamilies (G_s , G_i , G_q , and G_{12}) based on sequence and functional similarities. There are also five β and fourteen γ proteins. The α -subunit contains the guanine nucleotide binding site, whereas β and γ form a tightly associated $\beta\gamma$ -complex. When inactive, the α -subunit is bound to GDP and to $\beta\gamma$ -complex to form a trimeric protein complex. Agonist binding to the cell-surface GPCR activates the receptor, which then serves to both facilitate GDP release from and stimulate GTP binding to the α -subunit of coupled G proteins; that is, receptors are guanine nucleotide exchange factors for heterotrimeric G proteins. This GTP binding activates the α -subunit, leading to its dissociation from the $\beta\gamma$ -complex. Both α -GTP and $\beta\gamma$ can then bind to and activate intracellular effectors, such as second

messenger-generating enzymes as well as specific ion channels (Dickey & Birnbaumer 1993, Hall et al. 1999, Wickman & Clapham 1995). For example, the activated G_s - α proteins stimulate adenylyl cyclases, activated G_i - α proteins inhibit adenylyl cyclases, activated G_q - α proteins turn on phospholipase C- β , and activated G_{12} - α proteins stimulate guanine nucleotide exchange factors for the small GTP-binding protein Rho. The freed $\beta\gamma$ -subunits can activate or inhibit various adenylyl cyclases and activate phospholipase C- β and inward rectifying potassium channels (GIRK), among other effectors (Dickey & Birnbaumer 1993, Hall et al. 1999). Upon GTP hydrolysis, the GDP-bound α -subunit and the $\beta\gamma$ -subunits reassociate into the inactive G protein and cease activating the effector enzymes. GTP hydrolysis may occur through the intrinsic GTPase activity of the α -subunit or may be enhanced by the action of specific GTPase-activating proteins of the Regulators of G protein Signaling (RGS) family (Berman & Gilman 1998, Dohlmann & Thorner 1997, Neubig & Siderovski 2002) or by effectors themselves. Receptors vary in their specificity for activating or coupling to distinct G protein types, and thus activating downstream signaling pathways, with some receptor types activating only a single class of G protein to generate one class of intracellular signal, whereas other receptors more promiscuously couple to many G protein classes to generate multiple intracellular signals. Further, GPCRs may form homo- or heterodimers that could result in a complex variety of signaling events (Angers et al. 2002).

Mechanisms of GPCR Desensitization

One important feature of G protein signaling systems is that they are not constant but exhibit a memory of prior activation or signaling tone (Hausdorff et al. 1990). Thus, high activation of a receptor leads to a reduced ability to be stimulated in the future (desensitization), whereas low activation leads to an increased ability to be stimulated (sensitization). A given dose of agonist or drug thus may give distinctly different responses depending on the prior activation state of the system. This is an important regulatory feature that prevents overstimulation and allows for the linear response range to vary near the ambient stimulation level; in the visual system, such adaptation allows the G protein-coupled "light receptor" rhodopsin to adjust to both dark and light within moments.

GPCRs respond to activating ligands in a dose-dependent manner so that the concentration of agonist is the primary control point for signaling downstream of any given receptor. Receptors also differ in their basal or constitutive (that is, agonist-independent) activity, and in the extent of stimulation that a maximal dose of agonist can achieve. The ability of receptors to signal is regulated at the level of the receptor itself in two main ways: by controlling the number of receptors present on the cell surface and by regulating the signaling efficiency of receptors that are on the cell surface. Receptors are not static but are in equilibrium between cell-surface and endosomal pools and between synthesis and degradation. Receptor activation often leads to the removal of receptors from the cell surface by internalization,

and less often, to recruitment of new receptors to the cell surface. Internalized receptors can be recycled to the cell surface (resensitization) for further duty or targeted for degradation in lysosomes (downregulation). Prolonged stimulation generally leads to a profound receptor loss from the cell surface (Bohm et al. 1997).

One major mechanism controlling GPCR responsiveness is the activation-dependent regulation of receptors, also called homologous desensitization (Claing et al. 2002, Ferguson et al. 1998, Hausdorff et al. 1990, Lefkowitz 1998, Perry & Lefkowitz 2002, Sterne-Marr & Benovic 1995). This is discussed in detail below. Other mechanisms also contribute to intrinsic regulation of GPCR signaling (Bohm et al. 1997, Hamm & Gilchrist 1996, Watson & Arkininstall 1994). These include receptor activation-independent regulation of receptors, or heterologous desensitization, as well as mechanisms that act after the receptors themselves, through regulating the G proteins directly or by altering the signaling efficiency of downstream effectors. One common mechanism for heterologous desensitization is the feedback regulation of receptors by the second-messenger-regulated kinases they activate. For example, β -adrenergic receptors use Gs to activate adenylyl cyclase to synthesize cAMP, which activates protein kinase A (PKA). PKA can (among myriad other things) then phosphorylate the β -adrenergic receptors themselves, even those particular receptor proteins that were not activated by the current stimulation. PKA activated by stimulation of totally distinct receptor types can similarly phosphorylate and alter the responsiveness of β -adrenergic receptors. These PKA-phosphorylated receptors are less able to mount a response to a subsequent application of their own activating agonist. Similar regulation of various types of receptors occurs for GPCR-activated PKA, protein kinase C (PKC), mitogen-activated protein (MAP) kinases, and many other kinases. Along the same lines, second-messenger-activated kinase can also phosphorylate and regulate G protein effectors, such as adenylyl cyclase, phospholipase C, and others, also contributing to the cell's responsiveness to subsequent or concurrent activation (Hamm & Gilchrist 1996, Watson & Arkininstall 1994).

A distinct family of accessory proteins, the RGS proteins, act as GTPase-activating proteins (GAPs) for heterotrimeric G proteins (Berman & Gilman 1998, Dohlman & Thorner 1997, Neubig & Siderovski 2002). Thus, they promote inactivation of GTP-bound G protein α -subunits. The family of these proteins includes at least 25 members, all of which contain a characteristic RGS-homology domain consisting of about 130 amino acid residues. The physiological significance of RGSs in regulating GPCR signaling is still poorly characterized, but recent observations in knockout mice demonstrate the importance of this regulation for at least some brain functions (Rahman et al. 2003, Zachariou et al. 2003). Some G protein effectors also act partly as GAPs to promote G protein inactivation (Hall et al. 1999). Inasmuch as these RGS proteins and other GAPs can also be regulated by receptor activation, they will also contribute to altered signaling in response to prior signaling, and in any case will help to shape the basal responsiveness of the system by their mere presence.

GRKs and Arrestins in Homologous Desensitization

The activated state of GPCRs serves not only as an activator of G proteins, but also as the substrate for protein phosphorylation by a family of protein kinases called GPCR kinases (GRKs). GRKs can discriminate between the inactive and agonist-activated states of the receptor, in part because they are catalytically activated by stimulated receptors. Thus, activated receptor regulation by GRKs results in homologous desensitization (Figure 1) (Claing et al. 2002, Hausdorff et al. 1990, Lefkowitz 1998, Perry & Lefkowitz 2002, Sterne-Marr & Benovic 1995). There are seven known GRK subtypes, which are classified in three subfamilies (GRK1/7, GRK2/3, GRK4/5/6) based on sequence and functional similarity (Benovic et al. 1987, Chen et al. 1999a, Pitcher et al. 1998a, Premont et al. 1995, Willets et al. 2003). One of these families is primarily visual (GRK1/7), whereas one other kinase is expressed primarily in testes (GRK4). Thus four GRK subtypes (GRK2, GRK3, GRK5, GRK6) must account for regulation of most of the GPCRs found throughout the body (Gainetdinov et al. 2000, Pitcher et al. 1998a, Premont et al. 1995). All GRKs share a domain structure of an amino terminal RGS-like domain, a central protein kinase domain, and a variable carboxyl terminal. In the GRK2 subfamily, the RGS-like domain binds to G_q α -subunits but does not facilitate GTP

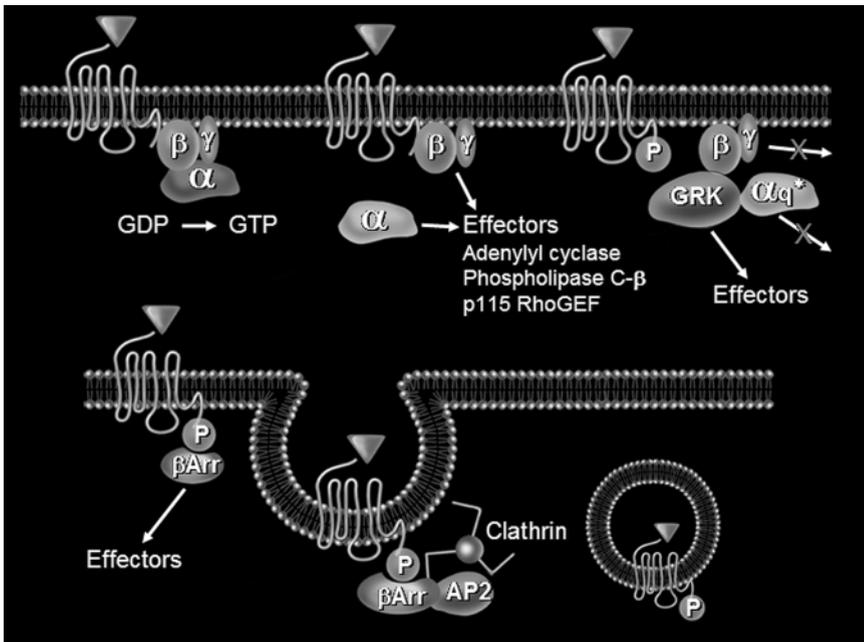


Figure 1 Schematic diagram representing key steps in GPCR signaling and homologous desensitization. See text for details.

hydrolysis (Pitcher et al. 1998a). Thus GRK2 acts not as a GAP but as a signaling dampener by preventing activated G_q α -subunit binding with other effectors, and perhaps by acting as a G_q effector itself. Other GRK subfamilies, though sharing a similar RGS-like domain, do not appear to bind G_q - α -subunits or other G proteins, and the function of this domain remains ill-defined (Pitcher et al. 1998a; Premont et al. 1994, 1996, 1999). In addition, the amino terminal domain has been implicated in recognizing activated receptors, although the recently solved structure of GRK2 tends to discount this (Lodowski et al. 2003). The GRK protein kinase catalytic domains are most similar to the PKA/PKC family (Hanks et al. 1988). The variable carboxyl terminal domains function in proper juxtamembrane localization of the GRKs (Pitcher et al. 1998a; Premont et al. 1995, 1999). In the GRK1 subfamily, the extreme carboxyl terminal is modified by prenylation, proteolysis, and carboxymethylation. GRK1 is farnesylated, whereas GRK7 is geranylgeranylated. In GRK1, the farnesyl group is critical for light-regulated membrane association: An unprenylated GRK1 fails to associate with membranes, whereas a geranylgeranylated GRK1 is constitutively membrane-associated. In the GRK2 subfamily, the carboxyl terminal domain is extended and contains a pleckstrin homology (PH) domain. The GRK2 PH domain binds to both PIP_2 and G protein $\beta\gamma$ -subunits. The $\beta\gamma$ -subunits, released from receptor-activated G proteins, and PIP_2 cooperate to activate the kinase. In the GRK4 subfamily, two types of carboxyl terminal membrane-association motifs are found, polybasic domains allowing PIP_2 binding, and in a subset (GRK4 and the GRK6A splice variant), palmitoylated cysteine residues (Stoffel et al. 1994, 1998). Importantly, GRK activity is a highly regulated process and may be determined not only by expression level and intrinsic activity, but also by subcellular compartmentalization of the kinase (Penn et al. 2000).

Once phosphorylated by a GRK, the activated receptor is bound by a member of another protein family, the arrestins (Figure 1). Arrestins recognize both GRK phosphorylation sites on the receptor and the active conformation of the receptor, so that both together drive robust arrestin association (Luttrell & Lefkowitz 2002, Perry & Lefkowitz 2002). Arrestins interdict further G protein activation despite the continued activation of the receptor by agonist by preventing the receptor from exchanging GTP for GDP on the G protein α -subunit. Arrestins consist of a bi-lobed predominantly β -sheet structure, with a large phosphoprotein binding pocket. The arrestin superfamily in vertebrates includes visual arrestins and β arrestins. Visual arrestins that play an important role in the modulation of phototransduction are expressed almost exclusively in the retina and represented by two members: rod arrestin (S-antigen or arrestin 1) and cone arrestin (CAR, X-arrestin, or arrestin 4) (Chen et al. 1999b). Intriguingly, rod arrestin was found also in pineal gland and in small populations of neurons in the brain, particularly in habenular commissura, amygdala, ventral tegmental area, and superior colliculus, which suggests that this arrestin may play some role in brain functions as well (Sunayashiki-Kusuzaki et al. 1997). However, the two nonvisual arrestins (β arrestins), β arrestin-1 (arrestin 2) and β arrestin-2 (arrestin 3), which are highly

expressed all over the body, must account for regulation of the vast majority of GPCRs.

In addition to this role as a receptor desensitization mechanism, the GRK-arrestin system also serves to promote the internalization of inactivated receptors and the subsequent recycling of resensitized receptors back to the cell surface (Ferguson et al. 1996, 1998). GRKs promote receptor internalization primarily by virtue of helping recruit arrestins to the activated receptors. The arrestins themselves bind to the clathrin adaptor protein AP2 and to clathrin itself, which facilitates the entry of desensitized receptors into clathrin-coated pits for subsequent internalization (Goodman et al. 1996, Laporte et al. 1999).

Furthermore, GRKs and arrestins appear to play direct signaling roles (Hall et al. 1999, Luttrell & Lefkowitz 2002). That is, along with the G proteins themselves, the GRKs and arrestins share the ability to recognize and bind to the activated state of the receptor. By virtue of binding to additional cellular proteins, GRKs and arrestins themselves serve as signal transducers by bringing specific signaling molecules into proximity of the activated receptor and the cell membrane and/or by altering their activity. Thus, GRKs have been reported to bind to GIT proteins and PI3-kinases and bring these to the receptor, where they are involved in regulating receptor trafficking and in promulgating further receptor-dependent signals (Hall et al. 1999). Arrestins serve as adaptors to ferry a wide variety of signaling proteins to activated receptors, including c-Src, entire MAP kinase cascades, Mdm2, ARNO, NSF, and others (Gurevich & Gurevich 2003, Luttrell & Lefkowitz 2002, Shenoy & Lefkowitz 2003). The relative contribution of these noncanonical signaling pathways as compared to direct G protein signaling in the CNS is mostly unknown but is an area of active investigation.

Specificity of GPCR Desensitization by GRKs and Arrestins

One major unanswered question regarding the physiological regulation of GPCRs is understanding which GRK(s) and arrestin(s) regulate any given receptor subtype. Studies over the past decade have defined the ability of certain GRKs and arrestins to phosphorylate and desensitize several GPCRs in model systems, but most receptors remain totally uncharacterized.

One approach to assessing the functional specificity of GRKs and arrestins *in vivo* is to ablate individual GRK or arrestin genes and determine whether this loss of function alters the regulatory properties of GPCRs. Over the past several years, we and others have developed mouse lines bearing deletions of each of the GRK and arrestin genes (Table 1 and references within), which we have used previously to characterize the GPCR regulation machinery in the heart (Rockman et al. 2002). In this way, we now can focus on other GPCR-mediated physiological systems of interest and assess whether any individual GRK or β arrestin (or combination of GRKs and β arrestins) plays an important role in regulating GPCR-mediated functions there.

TABLE 1 Expression pattern of GRKs and arrestins and major phenotypes in mutants lacking these proteins

GRK or arrestin	Expression	Knockout phenotype
GRK1 (Rhodopsin kinase)	Retinal rods and cones	Oguchi Stationary Night Blindness (human) (Yamamoto et al. 1997). Light-dependent retinal degeneration (mice) (Chen et al. 1999a).
GRK2 (β -adrenergic receptor kinase, β ARK; β ARK1)	Ubiquitous; brain	Embryonic lethal, thin myocardium syndrome in embryos (Jaber et al. 1996), and enhanced basal and adrenergic responses in cardiac function in adult heterozygotes (Rockman et al. 1998).
GRK3 (β -adrenergic receptor kinase 2, β ARK2)	Ubiquitous (in the brain lower than GRK2)	Lack olfactory receptor desensitization (Peppel et al. 1997). Altered M2 muscarinic airway regulation (Walker et al. 1999). Reduced tolerance to opioid fentanyl, but not morphine (Terman et al. 2004).
GRK4 (IT11)	Testes; brain (low)	Normal fertility and sperm function (R.T. Premont, unpublished). No obvious phenotype.
GRK5	Ubiquitous; brain	Altered central (Gainetdinov et al. 1999a) and lung (Walker et al. 2004) M2 muscarinic receptor regulation, with normal heart M2 receptor regulation (Walker et al. 2004).
GRK6	Ubiquitous; brain	Altered central dopamine receptor regulation (Gainetdinov et al. 2003a). Deficient lymphocyte chemotaxis (Fong et al. 2002). Increased neutrophil chemotaxis (Kaavelars et al. 2003, Vroon et al. 2004).
GRK7 (Iodopsin kinase)	Retinal cones	(Gene is not present in mice, but is present in humans)
Rod arrestin (S-antigen or arrestin 1)	Retinal rods	Oguchi Stationary Night Blindness (human) (Yamamoto et al. 1997). Light-dependent retinal degeneration (mice) (Chen et al. 1999b).
β arrestin-1 (arrestin 2)	Ubiquitous; brain	Altered cardiac responses to beta-adrenergic stimulation (Conner et al. 1997).

(Continued)

TABLE 1 (Continued)

GRK or arrestin	Expression	Knockout phenotype
β arrestin-2 (arrestin 3)	Ubiquitous (in the brain lower than β arrestin-1)	Enhanced morphine antinociception (Bohn et al. 1999, 2002) and reward (Bohn et al. 2003) and disrupted morphine tolerance (Bohn et al. 2000, 2002); deficient lymphocyte chemotaxis (Fong et al. 2002); impaired asthmatic response (Walker et al. 2003).
Cone arrestin (X-arrestin or arrestin 4)	Retinal cones; pineal gland	Not reported

These studies have demonstrated that loss of individual GRKs or β arrestins under basal conditions mostly produces relatively minor phenotypes, but in the presence of GPCR activators or other forms of stress, the importance of the GRK-arrestin regulation for GPCR is revealed (Table 1). A crucial role of visual rod arrestin and GRK1 in the termination of the light response in photoreceptors has been demonstrated convincingly in mice lacking these regulatory elements (Chen et al. 1999a,b). Among nonvisual GRKs, only GRK2 has proven to be embryonic lethal as a single gene deletion, owing to a developmental defect in the heart (Jaber et al. 1996). Loss of any other GRK or arrestin gene leads to a mouse that appears outwardly normal. Upon addition of exogenous GPCR agonists, however, abnormally supersensitive responses are present in some GRK knockouts, but not others, as measured by accentuated physiological responses. These supersensitive responses indicate that the GRK of interest is important for desensitizing that receptor type, at least in the tissue or system being examined.

Given the large number of GPCR types present throughout the body, only a fraction of GPCRs and receptor-regulated systems have been examined to date for regulation by GRKs and arrestins in any way. The relatively recent derivation of the last of the GRK knockout mouse lines means that the potential involvement of each GRK or arrestin has been examined in detail in vivo in very, very few systems. Our hope is that studies in the near future will begin to map the functional specificity of GRKs and arrestins in regulating many distinct, pharmacologically relevant GPCR types. One clear result of such studies is the realization that there exists a continuum of receptor regulation by GRKs and arrestins, such that some receptors appear to require one particular GRK or arrestin exclusively for their regulation, whereas other receptors are regulated in part by several GRKs or arrestins. Additionally, a given receptor need not be regulated by the same GRK or arrestin in all tissues.

Here, we summarize our experience in investigating the physiological roles and receptor specificity of neuronal GRKs and arrestins using knockout mice as a model. Particularly, results of initial screening for the role of each of these

molecules in classic physiological responses mediated by dopamine and μ -opioid GPCRs are presented, since receptors for dopamine (DA) and opiates are among the most clinically important neuronal GPCRs.

Dopamine and μ -Opioid Receptor–Mediated Behaviors as In Vivo Model Systems

Five distinct but related receptor proteins are activated by DA (dopamine D1–D5 receptors) and are classified into two distinct groups based on sequence and functional similarities: D1-like (D1 and D5) and D2-like (D2, D3, D4). Several signaling events can be regulated by DA receptors, including adenylyl cyclase and phospholipase C activity and the opening of various ion channels. The D1-like receptors couple to, or activate, the G_s family of G proteins (primarily G_{olf}) to increase cAMP production by adenylyl cyclase (mainly AC5) and are thought to be found only postsynaptically on dopaminergic target cells (in the striatum, primarily on GABA-ergic medium spiny neurons). The D2-like receptors couple primarily to the G_i family of G proteins to activate K^+ channels and inhibit adenylyl cyclase, and are present both presynaptically on DA-producing cells and postsynaptically on DA target cells. In the brain, the various receptor subtypes display specific distributions with highest density detected in the nigrostriatal and mesolimbic areas, such as caudate-putamen (striatum), nucleus accumbens, amygdala, and frontal cortex (Gardner et al. 2001, Grandy & Civelli 1992, Missale et al. 1998, Picetti et al. 1997, Schwartz et al. 1993, Seeman & Van Tol 1994, Sibley et al. 1999). DA plays a critical role in the control of movement, emotion, affect, and reward and is believed to be involved in brain disorders, such as Parkinson's disease, schizophrenia, addiction, Tourette's syndrome, attention deficit hyperactivity disorder (ADHD), and Huntington's disease (Carlsson 2001, Hornykiewicz 1966).

Cocaine and amphetamine are known to induce psychomotor activation by interfering primarily with the function of the dopamine transporter and thereby leading to elevated levels of DA in the extracellular space (Gainetdinov & Caron 2003b, Jones et al. 1998, Wise & Bozarth 1987). In rodents, the elevated DA levels in the major dopaminergic regions, such as striatum, are manifested behaviorally as locomotor hyperactivity. Sensitivity of DA receptors to endogenous and exogenous ligands is known to be an important modulator of DA-related functions. Supersensitivity of DA signaling has been suggested in human disorders such as schizophrenia (Jenner & Marsden 1987, Pandey et al. 1977), Tourette's syndrome (Singer 1994), and addiction (Hyman & Malenka 2001, Nestler & Aghajanian 1997, Nestler 2001, Robinson & Berridge 1993) and can be easily demonstrated in experimental animals chronically treated with psychostimulants (Laakso et al. 2002, Nestler 2001). This abnormal behavioral sensitization induced by chronic psychostimulants is associated with long-term changes in DA receptor responsiveness as evidenced by exaggerated locomotor responses not only to psychostimulants, but also to direct D1/D2 DA receptor agonists in various tests, such as the

characteristic “climbing” response to apomorphine (Wang et al. 1997, Wilcox et al. 1980). It is hypothesized that, among other mechanisms, long-term adaptations in GPCR desensitization can contribute to this phenomenon (Nestler & Aghajanian 1997, Nestler 2001), but the role of specific components of direct DA receptor regulatory mechanisms largely remains unknown.

Similarly, three distinct receptors (called μ , κ , and δ opiate receptors) bind opioid peptides and opiate drugs (Kieffer 1999, Snyder & Pasternak 2003). Each couples primarily to the G_i family of G proteins to activate K^+ channels and inhibit adenylyl cyclase and is found in many brain areas such as caudate-putamen, periaqueductal gray, thalamic nuclei, and amygdala. Further, each receptor is distributed differently throughout the CNS. Some areas express all three subtypes (striatum and dorsal horn of the spinal cord), whereas, for example, in the thalamic nuclei only μ -opioid receptor (μ OR) is found. Among the opioid receptors, μ OR is primarily involved in the antinociceptive activity, but it also has the highest abuse liability (DiChiara & North 1992). Several recent reports in genetically altered animals convincingly demonstrated the predominant role of μ OR in the antinociceptive and rewarding properties of morphine. Lack of morphine analgesia, as well as disrupted morphine-induced locomotor activity, hypothermia, respiratory suppression, gastrointestinal disturbances, tolerance to chronic treatment, dependence, and withdrawal, was observed in mice lacking the μ OR (Kieffer 1999, Loh et al. 1998, Matthes et al. 1996, Uhl et al. 1999). Therefore, the desensitization of the μ OR may present a critical point of regulation of the responsiveness to morphine that could dictate the extent of morphine effects on all of the physiological parameters, including the ones associated with its chronic use such as tolerance and addiction.

To understand the role of GRKs and β arrestins in DA receptor regulation and their contribution to aberrant neuroplasticity induced by chronic drugs of abuse, we have initially examined mice bearing inactivated GRK and arrestin genes for alterations in locomotor responses to cocaine, amphetamine, and/or nonselective dopamine agonist apomorphine. In a similar preliminary screen to assess μ OR responsiveness, the effect of morphine on centrally mediated analgesia was assessed in all these mutants using the classic hot-plate antinociception test as described (Bohn et al. 1999).

GRKS AND β ARRESTINS IN NEURONAL FUNCTIONS

Neuronal GRKs

GRK2 GRK2 was the first nonvisual GRK to be discovered, and it has been extensively characterized (Benovic et al. 1987, Pitcher et al. 1998a). The widespread expression of this kinase in many tissues in the body (Arriza et al. 1992) suggests that multiple GPCRs are physiological targets of this kinase. It is not surprising therefore that many GPCRs can be phosphorylated by GRK2 in *in vitro* preparations (Pitcher et al. 1998a, Premont et al. 1995). In the rat brain, GRK2 is expressed

in most neuronal populations, both in association with postsynaptic densities and presynaptically within axon terminals. Particularly, GRK2 immunoreactivity is found within cell bodies of neurons, as well as within structures that correspond to dendritic shafts, dendritic spines, and presynaptic axon terminals in most brain regions including those critical for locomotion and antinociception such as striatum, cortex, periaqueductal gray, and thalamus (Arriza et al. 1992). As with other GRKs, the pattern of expression of GRK2 does not correlate with that of any known single neurotransmitter system (Arriza et al. 1992, Erdtmann-Vourliotis et al. 2001). In a recent *in situ* hybridization study, GRK2 mRNA was found to be distributed in a nearly uniform manner through all cortical layers, the islands of Calleja, the claustrum, the dorsal endopiriform nucleus, the limbic diagonal band, the lateral septal nuclei, the bed nucleus of the stria terminalis, several hypothalamic and thalamic nuclei, hippocampus, the substantia nigra compacta, the ventral tegmental area, the pons, the reticulotegmental nucleus of the pons and the central gray, the cerebellar cortex, the locus coeruleus, and other regions. A significantly lower signal was detected in caudate-putamen (Erdtmann-Vourliotis et al. 2001). The expression levels of GRK2 display a marked increase during the second postpartum week in rat pups, reaching levels comparable to that in adult brain (Penela et al. 2000). GRK2 expression has been found to be altered in some disorders and can be modulated by pharmacological treatments. For example, a recent study reported that major depression may be associated with upregulation of GRK2 in the prefrontal cortex, and antidepressants appear to induce downregulation of the GRK2 protein (Grange-Midroit et al. 2003). Interestingly, acute but not chronic treatment with the norepinephrine transporter selective antidepressant desipramine (but not selective serotonin transporter inhibitor fluoxetine) increased membrane-associated GRK2-like immunoreactivity in the rat frontal cortex, which suggests that the *in vivo* activation of adrenergic receptors is associated with time-dependent modulation of GRK2 (Miralles et al. 2002). Responsiveness of hippocampal neurons to cannabinoid-mediated presynaptic inhibition of neurotransmission and luteinizing hormone secretion by pituitary gonadotropes is sensitive to GRK2 overexpression (Neill et al. 1999). One interesting aspect of GRK2 physiology that may have potential impact on brain functions is related to its ability to phosphorylate tubulin, thus potentially mediating GPCR effects on the neuronal cytoskeleton (Pitcher et al. 1998b). Furthermore, it has been reported that 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 (Pronin et al. 2000). One intriguing observation indicates that the neuronal calcium sensor-1 (NCS-1) can mediate desensitization of D2 DA receptors via interaction with GRK2 (Kabbani et al. 2002). Furthermore, NCS-1 was found to be elevated in the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder patients, which suggests that abnormalities in NCS-1-dependent desensitization of DA receptor signaling may contribute to these disorders (Koh et al. 2003).

In *in vitro* cellular systems, overexpressed GRK2 was shown to enhance phosphorylation and regulation of dopamine D1, D2, and D3 receptors (Ito et al. 1999,

Iwata et al. 1999, Kabbani et al. 2002, Kim et al. 2001, Lamey et al. 2002, Tiberi et al. 1996) and opioid receptors (Whistler & von Zastrow 1998, Zhang et al. 1998). Given the fact that this kinase is expressed in virtually all brain regions it is reasonable to expect that this regulation can occur *in vivo* as well. Deletion of the GRK2 gene in mice results in embryonic lethality due to cardiac hypoplasia (Jaber et al. 1996), so the role of this GRK on adult mouse behaviors cannot be examined fully. At the same time, mice heterozygous for this mutation are viable and do not display any obvious behavioral phenotype. These mice were used to characterize responses to psychostimulants and direct DA agonist apomorphine (Figure 2). As presented in Figure 2, doses of 10, 15, 25, and 30 mg/kg of cocaine induced comparable locomotor activation in wild-type and heterozygous mice, but at 20 mg/kg, cocaine induced significantly enhanced responses. Furthermore, no alterations were found when locomotor-stimulating effects to the indirect DA agonist amphetamine or climbing responses to the nonselective DA agonist apomorphine were analyzed. Thus, the impact of partial deletion of GRK2 on dopamine-mediated responses seems to be minimal. Nevertheless, the inavailability of GRK2 “null” mice does not allow us to exclude the involvement of this kinase in DA receptor regulation. Further studies would be necessary to examine this possibility in more detail.

Interestingly, it has been observed that GRK2 levels were increased in the locus coeruleus of rats chronically treated with morphine, suggesting a role of this kinase in μ OR regulation (Terwilliger et al. 1994). Furthermore, both acute and chronic treatment with opioid drugs as well as opioid withdrawal induce an increase in GRK2 levels in the rat cerebral cortex in experimental animals, and membrane-associated GRK2 levels are increased in brains of human opioid addicts (Ozaita et al. 1998). GRK2 immunoreactivity was increased in the cortex of rats treated with opioids and rendered tolerant to the antinociceptive effect of opioids (Hurlle 2001). Chronic treatment with the opioid antagonist naltrexone also resulted in significant upregulation of μ ORs, as well as several GRKs, including GRK2 (Diaz et al. 2002). In addition, it has been shown that GRK2 is highly expressed in nucleus raphe magnus GABAergic neurons projecting to spinal cord, where it appears to mediate desensitization of μ ORs (Li & Wang 2001). These and other (Fan et al. 2002) findings strongly suggest that GRK2 may contribute to the cellular processes underlying *in vivo* μ OR desensitization and could play an important role in the development of opioid tolerance and withdrawal. In our preliminary investigations, however, we did not see any significant difference in acute morphine-induced analgesia in the hot-plate test between GRK2 heterozygous and control mice (L.M. Bohn, unpublished). Again, further studies are necessary to explore this possibility more fully.

GRK3 The GRK3 protein shares a high structural similarity to GRK2, with over 80% amino acid identity (Arriza et al. 1992). In the periphery, GRK3 is highly expressed in olfactory receptor neurons and dorsal root ganglion (DRG) neurons and has been suggested to mediate homologous desensitization of odorant receptors in olfactory receptor cells and α_2 -adrenergic receptors in DRG neurons (Boekhoff et al. 1994, Diverse-Pierluissi et al. 1996). GRK3 mRNA and protein is widely

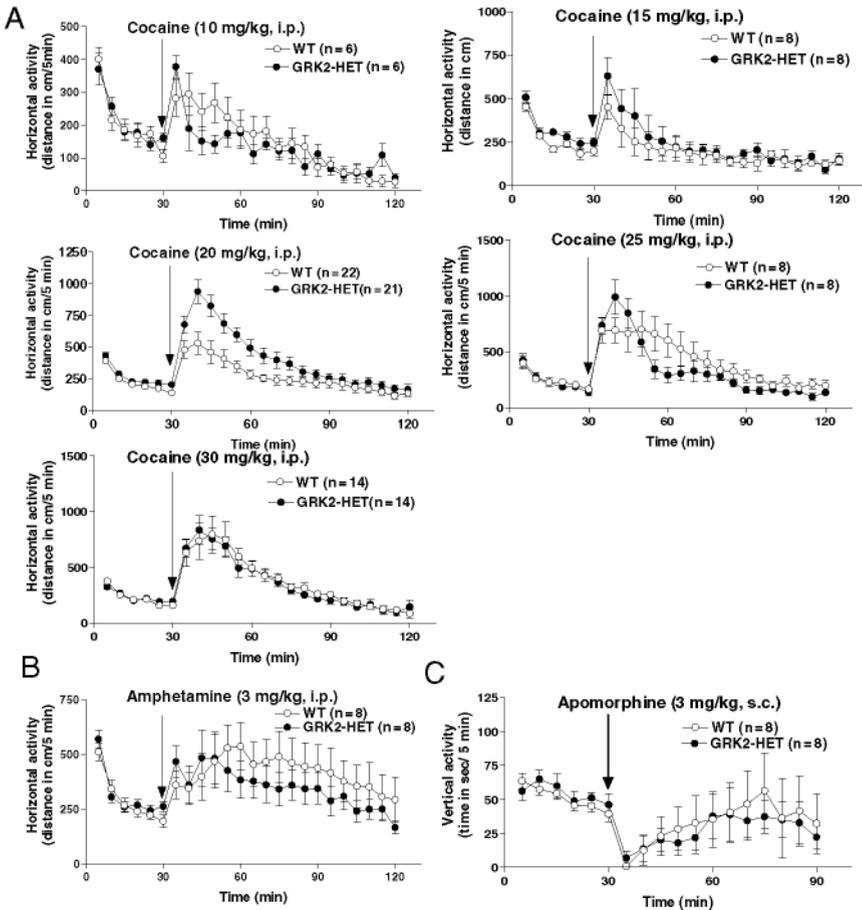


Figure 2 Locomotor responses of GRK2 heterozygous mice to (A) cocaine (10–30 mg/kg, i.p.), (B) amphetamine (3 mg/kg, i.p.) and (C) climbing responses to direct DA agonist apomorphine (3 mg/kg, s.c.). GRK2 heterozygous are significantly different from wild-type (WT) controls only in response to 20 mg/kg cocaine. GRK2 mutant mice (Jaber et al. 1996) were backcrossed 10 generations onto a C57/BL6 background (3–5 months old, both genders).

distributed in the rat brain, with a pattern of expression similar to GRK2, but at lower levels (Arriza et al. 1992, Erdtmann-Vourliotis et al. 2001). Interestingly, GRK3 mRNA was found at relatively low levels in the striatum, the paratenial thalamic nucleus, the bed nucleus of the stria terminalis, and in the periventricular hypothalamic nucleus. In contrast, GRK3 mRNA is expressed at levels higher than that of GRK2 in the islands of Calleja, the substantia nigra compacta, and in the locus coeruleus (Erdtmann-Vourliotis et al. 2001). In vitro studies in cell

culture have demonstrated a role of GRK3 in the regulation of numerous neuronal GPCRs, including D1, D2, and D3 dopamine receptors (Kabbani et al. 2002, Kim et al. 2001, Tiberi et al. 1996) and μ ORs (Cerver et al. 2001, Kovoor et al. 1998). A role for GRK3 in the adaptive changes in μ OR activity in the brain has been also suggested, based on alteration in expression of this kinase following opiate agonists and antagonists (Diaz et al. 2002, Hurle 2001). However, Terwilliger et al. (1994) reported that chronic morphine, while producing upregulation of GRK2 and β arrestin-1, failed to modify GRK3 levels in the rat locus coeruleus, and in another investigation expression of GRK3 remained unchanged after chronic treatment with opiates (Hurle 2001).

In a genome-wide linkage survey, the region of chromosome 22q12 containing the GRK3 gene was identified as a susceptibility locus for bipolar disorder in humans. Furthermore, GRK3 expression in the frontal cortex was found to be induced by amphetamine in the rats. Finally, transmission disequilibrium analyses indicated that two 5'-UTR/promoter polymorphisms are associated with human bipolar disorder, leading to the hypothesis that a dysregulation in GRK3 expression may alter GPCR desensitization, and thereby predispose affected individuals to the development of this disorder. It has also been suggested that primary candidates for this dysregulation would be DA receptors because DA has long been suspected to play an important role in bipolar disorder (Barrett et al. 2003).

Loss of the GRK3 gene leads to a mouse that appears outwardly normal but has impaired olfactory receptor desensitization and altered M2 muscarinic airway regulation (Peppel et al. 1997, Walker et al. 1999). In a basal locomotor activity test, these mice were not different from wild-type controls (Figure 3). Furthermore, mice lacking GRK3 do not demonstrate enhanced locomotor or climbing responses to either cocaine or apomorphine. In fact, each of these drugs induce somewhat reduced responses in mutant mice. Thus, it seems unlikely that this kinase is directly involved in regulation of dopamine receptors *in vivo*, at least for their effects on motor behaviors in mice. Rather, this kinase may impact other populations of GPCRs negatively affecting dopamine-related behaviors, such as, for example, receptors for serotonin (Gainetdinov et al. 1999b). Similarly, in a hot-plate analgesia test, acute morphine and fentanyl induced similar analgesia in both wild-type and GRK3-KO mice, but tolerance to the antinociceptive and electrophysiological effects of fentanyl was reduced in mutants. However, analgesic tolerance to morphine was not affected, which suggests that whereas GRK3 may play a role in opioid receptor regulation in response to high efficacy opioids, it is not the case regarding low-efficacy agonist morphine (Terman et al. 2004).

GRK4 The highest level of expression of GRK4 in the body is found in testes (Gainetdinov et al. 2000, Premont et al. 1996). Only a limited expression of GRK4 was detected in the brain, particularly in cerebellar Purkinje cells (Sallese et al. 2000). It has been demonstrated that the metabotropic glutamate 1 (mGlu1) receptor in cerebellar Purkinje cells can be regulated by GRK4, via a mechanism different from that used by GRK2 (Iacovelli et al. 2003). GRK4 may also play

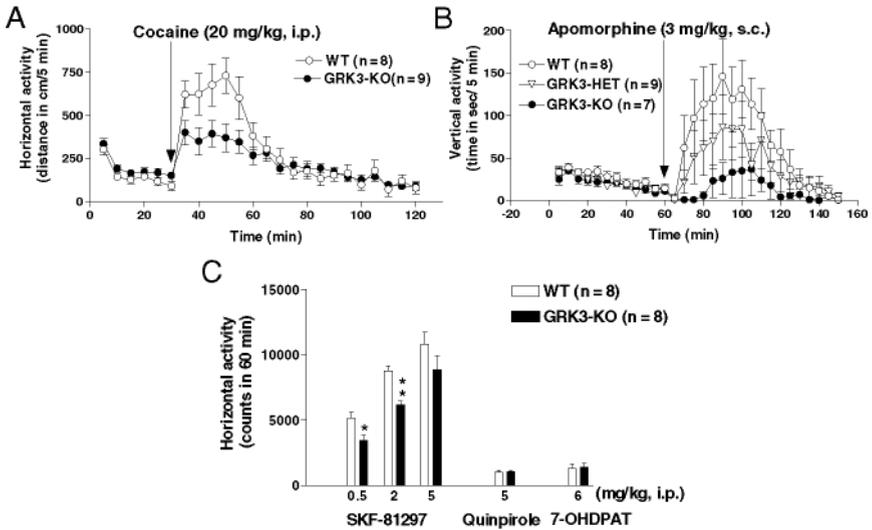


Figure 3 Responsiveness to cocaine (*A*) and direct DA agonists apomorphine (*B*), D1 DA agonist SKF-81,297 and D2/D3 agonists quinpirole and 7-OH-DPAT (*C*) in GRK3 mutant mice. *— $p < 0.05$ vs. respective WT controls. GRK3-KO mice are significantly less activated after cocaine, apomorphine, and SKF-81,297. GRK3 mutant mice (Peppel et al. 1997) were backcrossed 7 generations onto a C57/BL6 background (3–5 months old, both genders).

an important role in the agonist-promoted desensitization of GABA-B receptor in primary cerebellar granule cells (Perroy et al. 2003). These observations would suggest a role of GRK4 in motor coordination and learning (Sallese et al. 2000).

GRK4-KO mice showed no overt behavioral phenotype. No differences in basal level of locomotor activity or motor coordination in a rotorod test were found in these mutants. As might be expected from the expression pattern, locomotor responses to cocaine were not significantly changed in GRK4-KO mice (Figure 4). Furthermore, no difference in acute morphine-induced analgesia was observed in these mutants (L.M. Bohn, unpublished). Thus, the role of this kinase in the desensitization of brain DA receptors and μ ORs is unlikely. However, it is possible that GRK4 may be involved in desensitization of renal D1 DA receptors (Watanabe et al. 2002).

GRK5 GRK5 is the best-characterized member of the GRK4 subfamily of GRKs (Premont et al. 1995). The GRK5 mRNA is expressed widely in brain and in peripheral tissues, with highest expression evident in heart, lung, and placenta; however, the complete expression pattern of the GRK5 protein is still lacking. In the brain, GRK5 mRNA was found to be expressed moderately in several limbic regions such as the cingulate cortex, the septohippocampal nucleus, the anterior

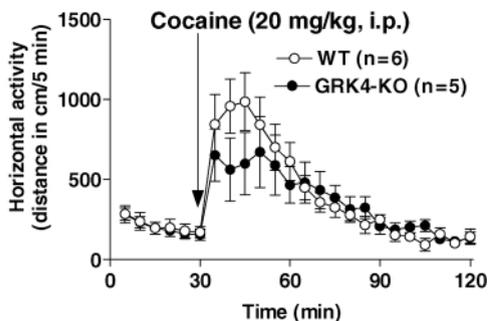


Figure 4 Locomotor responses of GRK4-KO mice to cocaine (20 mg/kg, i.p.) are not significantly changed. GRK4 mutant mice (generation of mice is described elsewhere; R.T. Premont & R.J. Lefkowitz, unpublished) were backcrossed 8 generations onto a C57/BL6 background (3 months old, both genders).

thalamic nuclei, the dentate gyrus of Ammon's horn, the medial habenula, the locus coeruleus, and the cerebellar cortex with the highest level of expression in the lateral septum (Erdtmann-Vourliotis et al. 2001).

In cellular model systems, GRK5 can phosphorylate several neuronal GPCRs, including β_2 -adrenergic, M_2 -muscarinic, secretin, angiotensin AT_1 , and thyroid-stimulating hormone receptors (Kunapuli et al. 1994, Menard et al. 1996, Pitcher et al. 1998a, Premont et al. 1994). D1 and D2 DA receptors (Ito et al. 1999, Tiberi et al. 1996) and μ OR (Koovor et al. 1998) can also be regulated by GRK5 in these model systems. Interestingly, investigators report that chronic, but not acute, treatment with cocaine resulted in upregulation of GRK5 mRNA in the septum (Erdtmann-Vourliotis et al. 2001). Furthermore, acute treatment with morphine, as well as spontaneous and naloxone-precipitated morphine withdrawal induced significant changes in GRK5 mRNA levels in several brain regions (Fan et al. 2002). One additional interesting aspect of GRK5 neurobiology arises from studies demonstrating the ability of different calcium sensor proteins and calmodulin to significantly modulate GRK5 activity (Iacovelli et al. 1999, Pronin et al. 1997). The calcium-dependent modulation of GRK5 may represent an important feedback mechanism to modulate homologous desensitization of these receptors in neuronal systems. Indeed, this may account for the curious observation that in transfected cell systems overexpressed GRK5 can phosphorylate and desensitize the angiotensin II AT_1 receptor, but transgenic mice with overexpression of GRK5 in myocytes exhibit normal heart contractility to angiotensin II (Rockman et al. 1996).

In mice lacking GRK5, only a very modest phenotype, a slight decrease in basal body temperature, was found (Gainetdinov et al. 1999a). Behavioral analyses were performed after challenging animals with a number of agonists to seek out the specific receptors affected by the loss of GRK5. We found no differences in cocaine-induced locomotor responses and climbing responses following a high

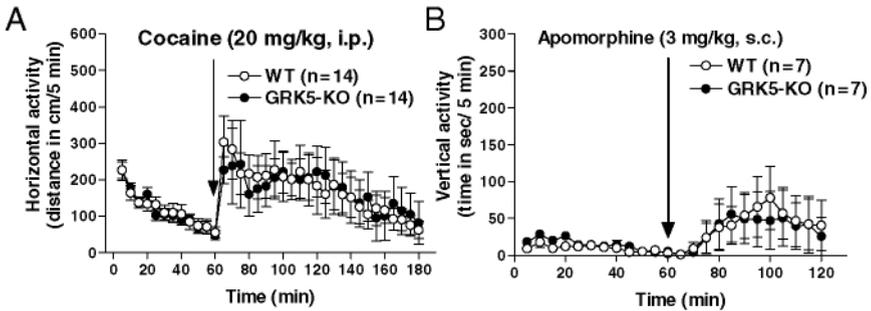


Figure 5 Responsiveness of GRK5-KO mice (Gainetdinov et al. 1999a) to cocaine (20 mg/kg, i.p.) and apomorphine (3 mg/kg, s.c.). Data on apomorphine responses are reproduced with permission from Gainetdinov et al. (1999a).

dose of apomorphine (Figure 5). Furthermore, acute morphine produced comparable analgesia in a hot-plate test in mutant and wild-type mice (L.M. Bohn, unpublished). Similarly, hypothermic responses to stimulation of serotonin 5-HT_{1A} receptor by 8-OH-DPAT did not differ between the genotypes (Gainetdinov et al. 1999a). These data suggest that the relative responsiveness of DA receptors, affected by cocaine and apomorphine, as well as the relevant μ ORs and 5-HT_{1A} subtype of serotonin receptors, seems to be unchanged by deletion of GRK5. At the same time, these mice demonstrated remarkable exaggeration of central muscarinic cholinergic responses (Gainetdinov et al. 1999a). The classic responses to muscarinic stimulation, such as hyperthermia, tremor, salivation, and locomotor suppression were all enhanced and prolonged in mutant mice. Further, the antinociceptive effect of nonselective muscarinic agonist oxotremorine was also significantly potentiated. The lack of oxotremorine-mediated desensitization of muscarinic receptors in membranes from the brain of GRK5-KO mice has been directly demonstrated using the ³⁵[S]GTP γ S binding assay. Although lack of selective agents precludes definitive clarification of the muscarinic receptor subtype involved, observations in transgenic animals strongly suggest that the vast majority of these behaviors are mediated by M2 muscarinic receptors (Gomez et al. 1999). The M2 receptor is widely expressed not only in the brain but also in many peripheral tissues and organs including smooth muscle and the heart. Indeed, recent studies suggest that M2 receptors in airway are supersensitive in the absence of GRK5, whereas M2 receptors in the heart are regulated normally (Walker et al. 2004). This demonstrates that the same receptor, M2 muscarinic in this case, need not be regulated by the same GRK in all tissues. The muscarinic-receptor-mediated hypersalivation response displayed by the GRK5 knockout mice (Gainetdinov et al. 1999a) suggests that M3 receptors are also probable targets for regulation by GRK5 in vivo (Wess 2000). Supersensitivity of muscarinic receptors has been described in several brain disorders, including depression and posttraumatic stress disorder,

as well as multiple chemical sensitivities (Janowsky et al. 1994, Overstreet et al. 1996, Sapolsky 1998). Moreover, rodent animal models of behavioral muscarinic supersensitivity have been developed to model these conditions (Overstreet et al. 1996). It would be of interest to explore the role of GRK5-mediated muscarinic receptor desensitization in these manifestations.

GRK6 GRK6 appears to be ubiquitously expressed (Benovic & Gomez 1993, Fehr et al. 1997, Gainetdinov et al. 2000). In the brain, GRK6 is expressed in many areas (Fehr et al. 1997) with the level and pattern of GRK6 mRNA expression somewhat similar to that of GRK2 mRNA. Interestingly, in the caudate putamen, GRK6 mRNA was highest of all GRKs (GRK2, GRK3, and GRK5), which suggests that GRK6 is the predominant receptor kinase in this brain area. Furthermore, GRK6 mRNA is also expressed in primary dopaminergic cell body areas, such as substantia nigra (Erdtmann-Vourliotis et al. 2001). In a recent immunohistochemical investigation (Gainetdinov et al. 2003a) a high expression of GRK6 protein was documented in the GABAergic medium spiny neurons as well as large cholinergic interneurons in the mouse striatum. These neurons represent the major striatal cell groups receiving dopaminergic input and are believed to be critically involved in brain disorders such as addiction, schizophrenia, and Huntington's disease.

GRK6 can regulate several neuronal GPCRs under in vitro conditions including D2 and D3 DA receptors (Gainetdinov et al. 2003a) and δ -opioid receptors (Willets & Kelly 2001). However, little is known about the role of this kinase in native brain tissue. Significant changes in the expression of GRK6 in rat brain were induced by chronic treatment with agonists and antagonists of μ ORs (Diaz et al. 2002, Hurler 2001), suggesting a role for this kinase in μ OR regulation. But, in the hot-plate morphine analgesia test, mice lacking GRK6 were indistinguishable from wild-type littermates (L.M. Bohn, unpublished). Like all the other GRK mutants available, mice lacking GRK6 under basal conditions do not demonstrate any obvious behavioral phenotype. However, GRK6-deficient mice, unlike other GRK mutants, are remarkably supersensitive to the locomotor-stimulating effect of psychostimulants (Gainetdinov et al. 2003a), including cocaine, amphetamine (Figure 6), and endogenous "trace amine" β -phenylethylamine (β -PEA) (Janssen et al. 1999, Premont et al. 2001). In biochemical experiments, these mice demonstrated an enhanced coupling of striatal D2-like DA receptors to G proteins and increased affinity for D2 but not D1 DA receptors. Furthermore, augmented locomotor response to direct dopamine agonists were observed both in intact and in dopamine-depleted animals (Figure 6). These data show that postsynaptic D2-like DA receptors are physiological targets for GRK6. These remarkably altered responses to cocaine and other psychostimulants suggest that a GRK6-dependent regulatory mechanism may contribute to central dopaminergic supersensitivity in various pathological states, such as addiction. One intriguing observation in these mice is that a 50% reduction in GRK6 levels in heterozygote mice produces a phenotype nearly identical to the complete knockout of the gene. This raises the possibility

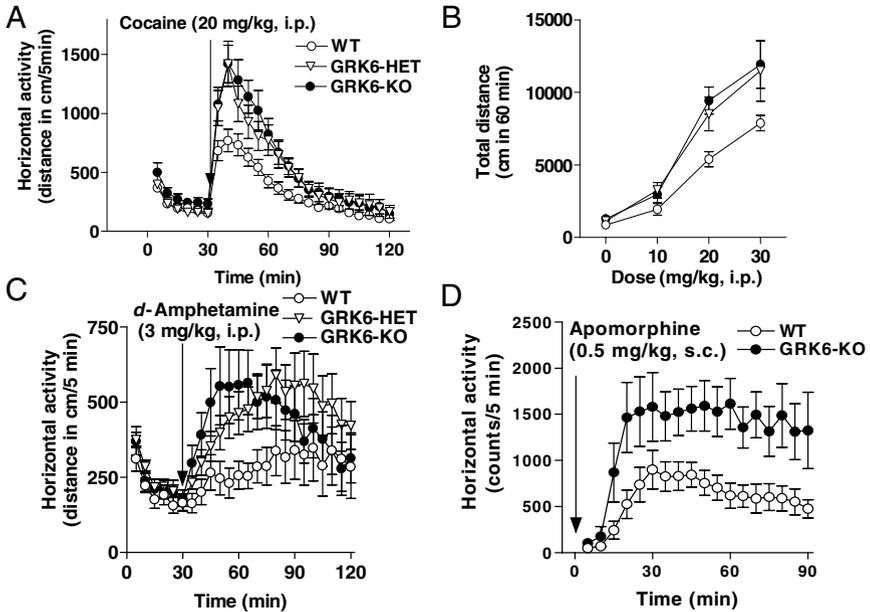


Figure 6 Enhanced locomotor effects of cocaine, amphetamine, and apomorphine in GRK6 heterozygous and knockout mice. (A) Dynamics of the effect of cocaine (20 mg/kg, i.p.) on the locomotion of mice. (B) Dose-response curve of the effect of cocaine (10–30 mg/kg, i.p.). Both GRK6 heterozygous and GRK6-KO mice are significantly different from WT controls in responses to cocaine. (C) Enhanced locomotor responses to amphetamine in GRK6 mutant mice. (D) Locomotor responses to direct DA receptor activation are enhanced in DA-depleted GRK6-KO mice. To deplete brain DA, animals were treated with a combination of reserpine (5 mg/kg, i.p.) and α -methyl-*p*-tyrosine (250 mg/kg, i.p.). Data are reproduced with permission from Gainetdinov et al. (2003a).

that even subtle allelic variations in the human GRK6 gene or drug-induced alterations in GRK6 expression or activity might contribute to individual sensitivity to drugs of abuse affecting DA function. In addition, supersensitivity to DA agonist stimulation in the absence or reduction of GRK6 suggests that a pharmacological strategy targeted on GRK6 expression or activity may be beneficial in conditions when dopaminergic signaling is limited, such as Parkinson's disorder. Because supersensitivity of DA receptors has been implicated in the pathogenesis or adverse reactions associated with treatment of schizophrenia, Tourette's syndrome, and Parkinson's disease (Jenner & Marsden 1987, Pandey et al. 1977, Singer 1994), a role for GRK6-mediated DA receptor regulation in these conditions should be considered.

β -Arrestins

β ARRESTIN-1 β arrestin-1 is expressed from birth in the majority of brain regions (Attramadal et al. 1992, Gurevich et al. 2002, Parruti et al. 1993, Penela et al. 2000). β arrestin-1 mRNA levels change during development, peaking on the fourteenth postnatal day and then somewhat decreasing, whereas protein levels continue to rise until adulthood (Gurevich et al. 2002). In adult rat brain, the highest expression of β arrestin-1 was detected in the cortex, hippocampus, striatum, and in the anterior, intralaminar, and midline nuclei of thalamus. In general, β arrestin-1 seems to be the major arrestin subtype in the rat brain. It has been estimated that in adult rat brain, the concentration of β arrestin-1 mRNA was two- to threefold higher than β arrestin-2 mRNA, whereas the ratio of β arrestin-1 to β arrestin-2 protein was much higher (10–20-fold) (Gurevich et al. 2002). Importantly, expression of β arrestins is particularly strong at postsynaptic densities but is also detectable at spines and nonsynaptic plasma membranes and intracellular organelles (Attramadal et al. 1992). In the spinal cord, immunoreactivity for β arrestin-1 was found in the motoneurons in lamina IX of the ventral horn and elongated cells in the dorsal nucleus of Clarke. Modest expression was detected in the neurons of laminae V and VII/VIII, and somewhat weaker immunoreactivity was observed in laminae III, IV, and X. Interestingly, in the spinal cord, both β arrestin-positive and -negative dendrites were observed, whereas axons and terminal boutons seem to be lacking β arrestins. Like in the brain, strong immunoreactivity for β arrestin-1 was mostly found at postsynaptic densities in the spinal cord (Kittel & Komori 1999).

In transfected cellular systems, β arrestin-1 appears to regulate desensitization of numerous GPCRs (Oakley et al. 2000), including D1 and D2 dopamine receptors (Kim et al. 2001, Oakley et al. 2000) and μ ORs (Oakley et al. 2000, Schulz et al. 1999, Zhang et al. 1998; but see Cheng et al. 1998). It was found that chronic systemic morphine treatment in rats produced an increase in β arrestin immunoreactivity in the locus coeruleus, measured using an antibody that recognizes both β arrestin-1 and β arrestin-2 (Terwilliger et al. 1994). In addition, chronic morphine treatment of cells expressing μ OR receptors resulted in attenuation of β arrestin-1 functions (Eisinger et al. 2002). In another investigation a differential regulation of β arrestin-1 mRNA in the locus coeruleus, periaqueductal gray, and cerebral cortex was observed following acute and systemic administration of morphine, but not during naloxone-precipitated withdrawal (Fan et al. 2003). Altogether, these findings suggest that β arrestin-1 may be important for at least some of the behavioral manifestations of acute and chronic morphine. It is important to note also that a presence of circulating autoantibodies reactive with β arrestin-1 has been described in multiple sclerosis patients (Ohguro et al. 1993).

Mice lacking β arrestin-1 demonstrate altered cardiac responses to β -adrenergic agonists, although these mice were found to be overtly normal (Conner et al. 1997). Analysis of basal locomotor activity also did not reveal any significant alterations in mutant mice (Figure 7). Furthermore, we did not observe the expected

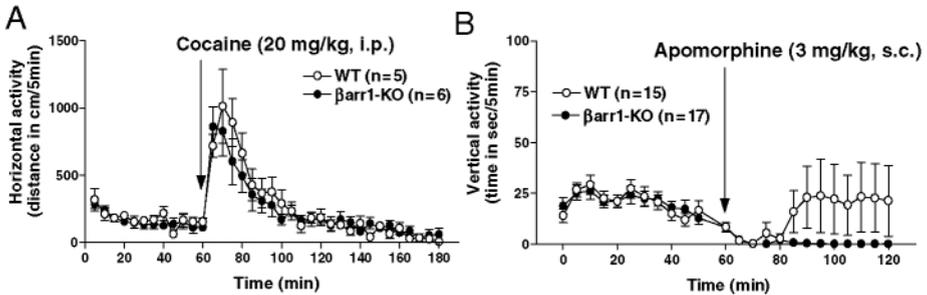


Figure 7 Responsiveness of β arrestin-1-KO mice to cocaine (20 mg/kg, i.p.) and apomorphine (3 mg/kg, s.c.). β arrestin-1-KO mice demonstrate significantly less climbing behavior after apomorphine. β arrestin1 mutant mice (Conner et al. 1997) were backcrossed 10 generations onto a C57/BL6 background (3–5 months old, both genders).

increase in dopamine-mediated locomotor responses. In fact, apomorphine-induced climbing was significantly suppressed (Figure 7). Surprisingly, we have observed no difference in acute morphine-induced antinociception in a hot-plate test in these mutants (L.M. Bohn, unpublished).

β ARRESTIN-2 β arrestin-2, like β arrestin-1, is widely expressed in many brain regions from birth (Attramadal et al. 1992, Gurevich et al. 2002). β arrestin-2 mRNA reaches maximal levels in neonatal pups and then somewhat decreases. During postnatal development, β arrestin-2 protein levels change little. In many brain areas there is a significant overlap between the expression of β arrestin-2 and β arrestin-1, suggesting colocalization of these proteins. However, each β arrestin also demonstrated a unique distribution in certain brain areas. Both arrestins were highly expressed in the cortex and hippocampus, but β arrestin-2 was particularly abundant in the medial habenular, in most hypothalamic nuclei, the extended amygdala, and, in the developing brain, in the subventricular zone (Gurevich et al. 2002). In the spinal cord, β arrestin-2 was found through laminae III–X in the order of IX > dorsal nucleus of Clarke > V > VII/VIII > IV > III > X (Kittel & Komori 1999).

Numerous GPCRs are known to interact with and be regulated by β arrestin-2 in transfected cell systems (Ferguson et al. 1998, Luttrell & Lefkowitz 2002, Perry & Lefkowitz 2002). β arrestin-2 in general appears to be more potent than β arrestin-1 in mediating agonist-dependent internalization and promotes faster recycling of many GPCRs, thus making this subtype the most commonly used in *in vitro* investigations of a GPCR desensitization. In cellular experiments, β arrestin-2 regulates D1, D2, and D3 DA receptors (Kim et al. 2001, Oakley et al. 2000, Zhang et al. 1999) and μ ORs (Bushell et al. 2002, Cerver et al. 2001, Kovoov et al. 1998, Lowe et al. 2002, Oakley et al. 2000, Whistler et al. 1999, Zhang et al. 1998). Interestingly, μ OR interaction with β arrestin-2 was found to be dependent on

agonist efficacy, and the low efficacy agonist morphine produced receptor desensitization at a significantly slower rate (Kovoor et al. 1998). However, overexpression of GRK2 or β arrestin resulted in robust morphine-triggered μ OR internalization and desensitization (Whistler & Von Zastrow 1998, Zhang et al. 1998), suggesting that despite substantial differences in the potency, morphine-activated μ ORs do not “elude desensitization by β arrestin” as suggested by Whistler & Von Zastrow (1998) but rather are subject to complex yet essential regulation by β arrestins.

A substantial amount of information has been gained on the role of β arrestin-2 in regulating receptor responsiveness to opioids. As discussed earlier, chronic morphine increases β arrestin-1/2 levels in the rat locus coeruleus (Terwilliger et al. 1994). Chronic treatment with μ OR agonist that resulted in a development of antinociceptive tolerance, as well as chronic μ OR antagonism, caused a significant increase in expression of β arrestin-2 in the cortex and striatum (Diaz et al. 2002, Hurlle 2001). Acute and chronic treatment with morphine variably regulates β arrestin-2 mRNA in several brain regions in rats (Fan et al. 2003). Finally, an involvement of β arrestin-2 in modulating spinal antinociception induced by μ OR agonists was demonstrated in mice recently by using intrathecally administered β arrestin-2 antibody (Ohsawa et al. 2003) or by using antisense RNA to β arrestin-2 (Przewlocka et al. 2002).

To assess directly if β arrestin-2 can be involved in regulation of μ ORs in physiologically relevant situations we assessed effects of morphine in mice lacking β arrestin-2. Mice lacking β arrestin-2 do not demonstrate any gross phenotype, although somewhat reduced locomotor activity in a novel environment is noticeable in these mice. However, when these mice were challenged with morphine, a number of characteristic reactions to this drug were remarkably altered. First, the acute antinociceptive effect of morphine in a hot-plate test is enhanced and prolonged in these mutants (Bohn et al. 1999, 2002). Importantly, alterations in the antinociceptive properties of morphine are correlated with enhanced μ OR-G protein coupling (Bohn et al. 1999, 2002). Second, tolerance to morphine's antinociceptive effects in this test is attenuated in β arrestin-2-KO mice (Bohn et al. 2000). Taken together, these studies demonstrated that lack of the β arrestin-2 leads to impaired desensitization of the μ OR, thus resulting in dramatically attenuated antinociceptive tolerance. Interestingly, manifestations of naltrexone-precipitated physical withdrawal were intact in β arrestin-2-KO mice, which suggests that, although the mutants did not experience antinociceptive tolerance to chronic morphine, they still became physically dependent on this drug (Bohn et al. 2000). It should be emphasized that these dramatic alterations in morphine analgesia in mice lacking β arrestin-2 were detected in the hot-plate test, a paradigm that primarily assesses supraspinal pain responsiveness. Morphine also induces spinal cord-mediated antinociception. In the warm water tail-immersion test, the paradigm primarily assessing spinal reflexes to painful thermal stimuli, the β arrestin-2-KO mice have shown greater basal nociceptive thresholds as well as markedly enhanced sensitivity to morphine. However, although they had a delayed onset of tolerance to chronic morphine in this test, the mutants eventually developed tolerance to this drug. Thus, in the absence

of β arrestin-2, the contribution of a previously elucidated PKC-dependent regulatory system to the development of morphine tolerance in this paradigm became apparent (Bohn et al. 2002).

Finally, significant alterations in locomotor and reinforcing properties of morphine were observed in β arrestin-2-KO mice (Bohn et al. 2003). The activation of both μ OR and DA receptors is known to play a critical role in the locomotor and reinforcing effects of morphine (Elmer et al. 2002, Kieffer 1999, Koob 1992, Maldonado et al. 1997). Morphine, as well as many other drugs of abuse, increases DA signaling in striatal and mesolimbic brain structures such as the striatum and the nucleus accumbens. In the case of morphine, stimulation of DA systems is indirect, originating from a disinhibition of GABAergic cells in DA cell body regions (substantia nigra and the ventral tegmental area) leading to increased neuronal firing and increased DA release in terminal regions (within striatum and nucleus accumbens) (DiChiara & North 1992). In mice lacking β arrestin-2, morphine produces a greater increase in DA release and induces increased reward as measured in conditioned place preference test. However, acute morphine treatment induced actually less pronounced hyperactivity in β arrestin-2 mutant mice, which potentially indicates an impact of this mutation on neurotransmitter systems other than DA, which are also involved in morphine effect on locomotion. Potential supersensitivity of these systems, such as serotonin (Sills & Fletcher 1997, Tao & Auerbach 1994), which are known to exert a general inhibitory action on DA-dependent hyperactivity (Gainetdinov et al. 1999b), may be involved and therefore needs further exploration. It is also interesting that in both wild-type and β arrestin-2 mutant mice chronic morphine induced comparable behavioral sensitization to this drug in locomotor test, suggesting a minimal role of β arrestin-2-mediated processes in this particular form of neuronal plasticity (Bohn et al. 2003).

In striking contrast to morphine, cocaine did not demonstrate exaggerated responses in these mice in any paradigm tested. In fact, acute cocaine induced somewhat reduced locomotor activation, but mutants demonstrated normal behavioral sensitization to chronic treatment. Similarly, a reduced response to direct DA

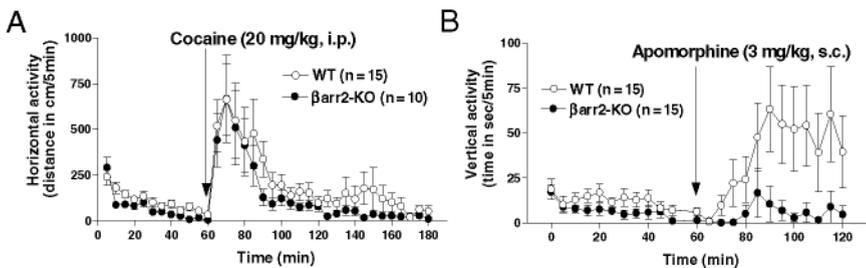


Figure 8 Responsiveness of β arrestin-2-KO mice (Bohn et al. 1999) to cocaine (20 mg/kg, i.p.) and apomorphine (3 mg/kg, s.c.). β arrestin-2-KO mice demonstrate lower basal activities and significantly less climbing after apomorphine. Data on cocaine responses are reproduced with permission from Bohn et al. (2003).

agonist apomorphine was noted (Figure 8). At the same time, cocaine induces normal elevation in extracellular DA and reward in conditioned place preference. Thus it is likely that β arrestin-2 is not critically involved in the regulation of DA receptor desensitization. However, a reduced basal locomotion, as well as responses to cocaine and apomorphine, may suggest that without this regulatory element, DA signaling may be somewhat impaired.

CONCLUSIONS

Seven GRKs and four arrestins have been identified, whereas more than 700 GPCRs have already been cloned (Luttrell & Lefkowitz 2002, Perry & Lefkowitz 2002, Pitcher et al. 1998a, Wise et al. 2004). Thus, redundancy in the mechanisms of GPCR regulation, such as the involvement of each GRK or arrestin in the desensitization of multiple receptors, is to be expected. Most of the initial work attempting to define the specificity of GRKs' and arrestins' action on individual GPCRs has used either overexpression of these proteins in cultured cells or purified GRKs acting on receptor preparations (e.g., in washed rod outer segment membranes or purified receptor proteins reconstituted into lipid vesicles). Other *in vitro* studies have attempted to assess inhibition of individual GRKs (using heparin or anti-GRK antibodies or expressing GRK fragments or kinase-deficient mutants), but generally they have suffered from incomplete inhibition or uncertainty about the specificity of inhibition. These studies have revealed significant differences in the substrate recognition of GPCRs among the GRK subtypes (Pitcher et al. 1998a). Similarly the specificity of interaction of arrestin family members with GPCRs in cultured cells has been demonstrated using green fluorescent protein-tagged arrestins. In these *in vitro* studies, analysis of agonist-mediated arrestin translocation to multiple GPCRs identified two major classes of receptors (Class A and Class B), which differ in relative affinity for β arrestin-1 versus β arrestin-2 and in internalization/recycling properties (Oakley et al. 2000). However, whether this specificity could be demonstrated in native tissue *in vivo* has largely remained unknown. The development of mice lacking specific GRKs or arrestins brings yet a novel tool to address the problem of matching individual GRKs and arrestins with specific GPCRs and the physiology they control.

The observations summarized here demonstrate the functionally important roles of specific GRKs and β arrestins in the regulation of DA and μ -opioid receptors. However, although this initial screening brought to our attention a prominent role of GRK6 in DA-mediated responses and β arrestin-2 in μ OR-mediated responses, many questions remain unanswered.

With regard to regulation of DA receptors (Table 2), one important question remains: Which particular receptor subtypes are primarily regulated by which GRK and β arrestin? Functionally, GRK6 clearly appears to be the main GRK regulator of some D2-like receptor, either D2 or D3, or perhaps both. Although assessment of binding characteristics of striatal DA receptors and locomotor responses to

TABLE 2 Summary of locomotor responses to psychostimulants and dopaminergic agonists in mice lacking GRKs and β arrestins

Mutants	Basal level	Cocaine	Apomorphine	Other drugs
GRK2-HET	Normal	Slightly increased	Normal	Normal response to amphetamine
GRK3-KO	Normal	Decreased	Decreased	Decreased response to D1 dopamine agonist
GRK4-KO	Normal	Not different	Not tested	Not tested
GRK5-KO	Normal	Not different	Normal	Not tested
GRK6-HET	Normal	Markedly increased	Increased	Increased responses to amphetamine; β -PEA, D2 DA agonist;
GRK6-KO				Normal responses to D1 DA agonist
β arrestin-1-KO	Normal	Not different	Decreased	Not tested
β arrestin-2-KO	Decreased	Not different	Decreased	Decreased responses to morphine

selective agonists demonstrated that properties of D2-like DA receptors, but not that of D1-like, are significantly affected in GRK6 mutant mice, the possibility that various other populations of DA receptors might also be regulated by GRK6 cannot be excluded. It would be reasonable to expect also that more than one GRK would be needed for regulation of all five subtypes of DA receptors; however, in our initial screen no obvious alterations in DA responses were found in other GRK mutants. D1-like receptors appear subject to GRK regulation in transfected cell systems, yet no single GRK appears to regulate these receptors in knockout mice. Perhaps D1 can be regulated by several GRKs such that loss of any one does not perturb its regulation significantly. At the same time, the minor alterations found in GRK2 heterozygous mice in locomotor responses to cocaine leaves this GRK as a suspect and suggests that further investigation is needed to test a role for GRK2 in DA signaling. Also important, lack of behavioral alterations in responses to psychostimulants does not necessarily mean that no DA receptors are affected by these mutations. It is also possible that there may be functional substitutes for the missing GRK that allow the animal to maintain a normal phenotype. For example, other downstream signaling elements such as PKA may be involved in regulating the receptors (Hausdorff et al. 1990). Alternatively, RGS proteins, particularly the subtype highly enriched in the striatum, RGS9-2, also could play a role in direct modulation of DA receptor function. RGS proteins are known to accelerate the rate of GTP hydrolysis on activated G proteins, so they can speed up the

deactivation of a system after stimulation or dampen signaling during persistent stimulation. In fact, recent observations of a functional interaction between RGS9-2 and D2 DA receptor signaling and the behavioral effects of psychostimulants suggest that psychostimulants may induce RGS9-2 to diminish responsiveness to a drug (Rahman et al. 2003).

Even more intriguing, in this initial screening, no supersensitivity to DA stimulation was found in either the β arrestin-1 or β arrestin-2 mutant mice (Table 2). Furthermore, even mice developed by crossing these two strains, three-allele knockouts, β arrestin-1-KO/ β arrestin-2+/- and β arrestin-1+/-/ β arrestin-2-KO mice, behave similarly to single knockout lines; that is, they showed somewhat reduced responses to apomorphine (R.R. Gainetdinov, unpublished). An attractive hypothesis is that there is significant functional redundancy between the two β arrestin subtypes so that the complement of one isoform of β arrestin is sufficient to regulate dopamine receptors in the absence of the other isoform. In fact, in transfected cell-culture systems, D1 and D2 DA receptors do interact with both types of β arrestins (Kim et al. 2001, Oakley et al. 2000), and expression of both β arrestins substantially overlaps in primary DA regions. It is also notable that lack of both β arrestin-1 and β arrestin-2 is embryonically lethal in mice (Kohout et al. 2001), suggesting a critical and cooperative role of both these regulatory proteins for normal physiological functions, at least during development. In line with this conclusion, β arrestin-2 appears to be involved in the Wnt5A-stimulated endocytosis of Frizzled 4 (Chen et al. 2003), indicating a direct role of this multifunctional adaptor protein in the regulation of developmental pathways. Further, although we have assessed dopaminergic stimulant function with single drugs, intact animals possess a myriad of other receptors and agonists that may also be subject to regulation by arrestins, and it may be that compensatory alterations in other receptor pathways may mask β arrestin effects on the DA receptors themselves. Alternatively, it is possible that the recently characterized signaling properties of β arrestins (Luttrell & Lefkowitz 2002) may be functionally relevant for DA signaling, and inactivation of β arrestins may contribute directly to a reduction in DA-dependent responses observed in both β arrestin mutant strains.

Similar considerations also should be taken into account when effects of morphine were analyzed in mutants lacking GRKs or β arrestins. β arrestin-2-KO mice have demonstrated remarkable alterations in μ OR regulation and morphine responses not seen with β arrestin-1 deletion. Surprisingly, no GRK-KO mice available demonstrated altered antinociceptive responses to morphine, comparable to that found in β arrestin-2 mutants. It is, thus, possible that more than one GRK may be involved in μ OR regulation, and other subtypes compensate for lack of any specific GRK. Importantly, the apparent specificity of desensitization of a particular GPCR measured *in vitro* does not seem to be an accurate predictor of specificity in the intact animal. For example, to demonstrate a role of β arrestin-2 in morphine-induced desensitization of μ ORs in cellular model systems, overexpression of GRK2 was necessary (Zhang et al. 1998), but in mice lacking β arrestin-2 virtually all physiological responses to morphine are remarkably altered. Among

the primary determinants of GPCR-GRK-arrestin specificity, not only intrinsic kinase activity but also the colocalization of the receptor, kinase, and arrestin within individual cells in a tissue or brain region are critical. One expectation is that this specificity will vary considerably, with some receptors being regulated by a single GRK subtype without compensation by other GRKs, whereas other receptors might be regulated more-or-less equally by several GRK/arrestin subtypes. The μ OR would appear to be an example of a receptor that can be regulated by several GRKs, such that loss of any one does not appreciably alter receptor function. However, the ability of other GRK subtypes to compensate for the loss of one GRK subtype can be limited, at least for some receptors, as evidenced by the embryonic lethality of the GRK2 knockout mouse. Again, we cannot completely exclude that GRK2 is primarily involved in this regulation and future development of time or region-selective GRK2 mutants would be helpful to address this issue. Interestingly, chronic morphine differentially affects various neuronal populations; that is, some populations display μ OR desensitization, whereas other brain regions do not (Noble & Cox 1996, Sim et al. 1996). Furthermore, it has been demonstrated by Haberstock-Debic et al. (2003) that morphine can induce μ OR internalization in dendrites but not cell bodies of neurons of the nucleus accumbens, hinting that receptors residing in different compartments within the same neurons may be subject to different means or degrees of regulation. This could, for example, depend on the cellular complement and fine localization of regulatory elements.

Our findings thus far suggest that there is significant degree of specificity in the regulation of the specific GPCRs by GRKs and β arrestins *in vivo*. Although we have utilized simple screens to assess the most obvious differences between genotypes in response to morphine or cocaine, we do not eliminate the possibility that we may be overlooking changes in responsiveness in other behavioral paradigms. For example, whereas the β arrestin-2-KO mice are supersensitive to morphine in antinociception tests, they are actually less activated in locomotion by morphine (Bohn et al. 2003).

The observations summarized here strongly support the general principle that the inactivation of components of GRK/arrestin-mediated desensitization machinery leads to enhanced GPCR signaling, and thereby, enhanced physiological function mediated by a given receptor (Bohn et al. 1999, 2002, 2003; Gainetdinov et al. 1999a, 2003a). The challenge remaining is to extend this principle to the vast sea of remaining receptors and the physiological responses they control. A further indication of the degree of importance of the desensitization machinery in modulating physiological responsiveness is the fact that both the GRK6 and the β arrestin-2 heterozygous mice demonstrate alterations almost identical to that observed in mice lacking these proteins (Bohn et al. 1999, 2002, 2003; Gainetdinov et al. 2003a). Therefore, deficiency in one allele, or pharmacological inhibition of half of the GRK6 or β arrestin-2 activity, could potentially result in a maximally possible physiological phenotype similar to that observed upon full inactivation of the protein. This could have great therapeutic implications because most pharmacological inhibitors do not result in complete elimination of molecular function.

One brain disorder where GPCR desensitization processes could be particularly important is addiction, where abnormal neuronal plasticity to chronic drug treatment is believed to be a primary cause for compulsive drug abuse. Among several behavioral manifestations of these permanent molecular changes, such phenomena as “sensitization” and “tolerance” directly point to the alterations in sensitivity of a receptor to its agonists. Repeated administration of psychostimulants like cocaine is known to result in a progressive enhancement of psychomotor responses, and this paradigm is classically used in studies of drugs of abuse in experimental animals. This phenomenon, termed behavioral sensitization or reverse tolerance, is believed to be related to permanent neuronal adaptations associated with alterations in responsiveness to DA stimulation (Hyman & Malenka 2001, Laakso et al. 2002, Nestler 2001). The abnormal supersensitivity of DA receptors involves changes in signaling molecules such as transcription factors Δ FosB and the cyclic-AMP response-element-binding protein (CREB), as well as cyclin-dependent kinase 5 (Cdk5) (Bibb et al. 2001, Hyman & Malenka 2001, Nestler 2001). However, the contribution of direct DA receptor regulatory mechanisms in this phenomenon has only begun to be characterized (Gainetdinov et al. 2003a; Rahman et al. 2003). As summarized in the present review, we find that direct regulation of DA receptors by GRK6, but not by other GPCR specific kinases, represents an important determinant by which receptor supersensitivity and responses to drugs of abuse can be controlled. This raises an intriguing possibility that potential alterations in GRK6 levels or activity could be another important determinant of responsiveness to drugs of abuse.

Strikingly, an opposite reaction to chronic drug treatment, termed tolerance and defined as a decrease in the biological effect of the drug despite a constant dose, has also been found affected in mice deficient in homologous desensitization. Particularly, in the best-known paradigm to study this process, morphine-induced antinociceptive tolerance, β arrestin-2-KO mice were deficient while retaining classic withdrawal reactions to chronic morphine. Moreover, it has been observed that morphine is more rewarding to the β arrestin-2-KO mice than to wild-type mice, an observation that correlates with their pain responses (Bohn et al. 2003). It will be very important to further assess the contribution of each of these regulatory elements, specifically, to the development of behaviors associated more directly with drug abuse. Future testing of the GRK and β arrestin KO mice in paradigms that assess drug reinforcement, such as conditioned place preference and self administration, should further our knowledge of the contribution of GPCR regulation to the molecular mechanisms of neuronal plasticity underlying drug addiction.

From this initial characterization of the GRK and β arrestin mutants, it has become evident that under basal, unchallenged conditions, these mice generally demonstrate only a modest phenotype, if any at all. This observation of a weak phenotype suggests that these regulatory elements play a minor role in setting the basal “tone” of the signaling pathway. However, upon challenge with the relevant agonist, a GPCR that cannot be properly regulated results in a mutant animal that

is no longer able to compensate to the same degree as wild-type controls. Thus, it is reasonable to expect that a potential pharmacological window of future drugs targeting desensitization machinery would be related to the fine tuning of physiological responses. Having mice available that lack each GRK/arrestin subtype will now permit examination of any individual GPCR and its physiological effects to assess the contribution of each GRK or arrestin to regulation of that receptor. In addition, because altered responsiveness of GPCRs has been found in many diseases, the use of mutant mice will allow evaluation of the potential contribution of these molecules to these conditions. Knowledge of this sort will be critical to promote further specific GRKs or arrestins as potential therapeutic targets. Furthermore, an important and very specific role of GRKs and β arrestins in complex functions, such as locomotion and antinociception, suggests that a precise and safe pharmacology based on targeting these general kinases and arrestins is possible. As one such example, it is conceivable that pharmacological inactivation of GRK6 may provide an effective approach to amplify efficacy of endogenous or exogenous DA stimulation and may be helpful to restore movement in patients with Parkinson's disease. Similarly, suppression or inhibition of β arrestin-2 may become an effective strategy to enhance antinociceptive properties of the "gold standard" analgesic morphine without affecting, or actually reducing, its side effects. Thus, targeting GPCR desensitization machinery may provide a novel principle of pharmacology where fine tuning of GPCR signaling, rather than direct receptor stimulation or blockade, would result in a more precise correction of abnormal physiological processes.

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