Modulation of Monoamine Receptors by Adaptor Proteins and Lipid Rafts: Role in Some Effects of Centrally Acting Drugs and Therapeutic Agents

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Annu. Rev. Pharmacol. Toxicol. 2011. 51:211–42

First published online as a Review in Advance on September 27, 2010

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev-pharmtox-010510-100520

0362-1642/11/0210-0211$20.00

Keywords

dopamine, serotonin, GPCR, caveolin, scaffolding proteins, ligand bias

Abstract

The monoamines and their cognate receptors are widespread in the central nervous system and are vital for normal brain function. Dysfunction in these systems underlies several psychiatric and neurological disease states, and consequently monoamines are targets of a host of pharmacotherapies. This review provides an overview on how monoamine receptors are regulated by adaptor proteins and lipid rafts with emphasis on interactions in nerve cells. Monoamine receptors have prominent intracellular loops that provide binding sites for adaptor proteins. Receptor function is further modulated by cholesterol and submembranous microdomains termed lipid rafts. These interactions determine several facets of G protein–coupled receptor (GPCR) function including trafficking, localization, and signaling. Possible roles of adaptor proteins and lipid rafts in disease states and in mediating actions of drugs and therapeutic agents are also discussed.

INTRODUCTION

Monoamines are derived from aromatic amino acids such as phenylalanine, tyrosine, tryptophan, and the thyroid hormones by the action of the aromatic amino acid decarboxylase. Monoamines include serotonin, dopamine, noradrenaline, adrenaline, histamine, and trace amines, and they are widespread in the nervous system and critical for proper emotional, cognitive, and motoric responses and execution. Monoamine neurotransmission is dysregulated in numerous disease states of the central nervous system (CNS) including schizophrenia, depression, drug addiction, and Parkinson's disease and is targeted by multifarious agents for the treatment of these conditions. Almost all actions of monoamines are mediated through G protein–coupled receptors (GPCRs), which are seven-transmembrane receptors with intracellular loops that enable protein-protein interactions (1, 2). Some GPCRs are monomeric, but it has become evident that they often form dimers and heteromers with other GPCRs, ionotropic receptors, and ion channels. The importance of dimeric and heteromeric interactions among GPCRs has been thoroughly reviewed elsewhere (3, 4) and is not addressed here.

GPCRs comprise the largest family of transmembrane proteins in vertebrates, and they are the molecular targets for nearly half of the therapeutic drugs that are prescribed worldwide (5). The established and traditional view is that stimulated GPCRs act as guanine nucleotide exchange factors for α subunits of heterotrimeric G proteins, facilitating the release of GDP and the binding of GTP, resulting in G protein activation. The activated G protein subunits (α, β, γ) then dissociate and, via downstream effector molecules, modulate different aspects of cellular physiology. Agonist stimulation of GPCRs promotes interactions with GPCR kinases (GRKs), leading to receptor phosphorylation and recruitment of arrestins. The interaction of GPCRs with arrestins results in desensitization of the GPCRs through steric inhibition of G protein interaction, and promotion of receptor internalization via clathrin-dependent mechanisms (6, 7).

GPCRs also bind to a multitude of intracellular adaptor proteins, which determine surface expression and anchoring, the internalization process, and the G protein–dependent and –independent signaling of the receptor (see sidebar, Adaptor Proteins). In some instances, the regional, cellular, and/or subcellular distribution of an adaptor protein provides cell-type specificity of GPCR responses. Moreover, some adaptor proteins are inducible; i.e., they exhibit marked differences in expression levels during disease states and after various stimuli including drug treatments (see below). The number of adaptor proteins that have been shown to interact with monoamine receptors is large (see **Table 1**) and is rapidly expanding. It is beyond the scope of this review to describe in detail all these interactions, and, for some of them, more detailed information has recently been published elsewhere (8–13). Here we focus on a few adaptor proteins whose interactions with adrenergic, dopaminergic, and serotonergic (5-HT) receptors illustrate principles that might have profound effects on receptor functionality.

ADAPTOR PROTEINS

Adaptor proteins interact with the intracellular regions of GPCRs and determine signaling and trafficking of the receptor. They may provide cell-type specificity of GPCR responses. Some adaptor proteins are inducible; i.e., they exhibit marked differences in expression levels during disease states and after various stimuli including drug treatments.

Table 1 Adaptor proteins interacting with monoaminergic receptors

Receptor	Adaptor protein	Reference
α_{1A}-AR	Snapin	155
α_{1B}-AR	Ezrin	156
	AP1M2	157
	Spinophilin	50, 158
α_{1D}-AR	Syntrophin	159
α_{2A}-AR	Uch-L1	160
α_{2A}-AR	Spinophilin	53
α_{2B}-AR	Spinophilin	52
β_1-AR	AKAP79 (AKAP5)	161
	PSD-95	27
	GIPC	162
	Endophilins	163
	MAGI-2	164
	Rapgef2	165
	SAP97, MAGI-3	166
β_2-AR	AKAP79 (AKAP5)	167
	AKAP250 (AKAP12, gravin)	168
	NHERF1	32
	NSF	35
	eIF-2B	169
	Grb2	170
	Src	171
β_3-AR	Src	172
D1	Drip78	40
	AIP1	173
	Neurofilament-M	174
	Calnexin	41
	γ-COP	175
	PSD-95	176
	Cav-1	88
	β-arrestin 1 (Arrb1), β-arrestin 2 (Arrb2)	177
	NSF	34
	MAGI-2	173
D2	Calmodulin	178
	Spinophilin	49
	CAPS1	179
	GASP1	180
	NCS-1, GRK2	55
	GIPC	42
	4.1N, 4.1B, 4.1G	181
	β-arrestin 1 (Arrb1), β-arrestin 2 (Arrb2)	182
	Calnexin	41
	Dynamin-2	183
	Filamin-A	184

(*Continued*)

Table 1 (*Continued*)

Receptor	Adaptor protein	Reference
	H-FABP	185
	Par4	147
	GAIP	42
	NSF	186
	ZIP	187
	S100B	57
D3	NCS-1	55
	4.1N, 4.1B, 4.1G	181
	Grb2	188
	β-arrestin 2 (Arrb2), Filamin-A	189
	AIP1	173
	CLIC6	190
	GIPC	42
D4	Nck, Grb2	191
D5	Neurofilament-M	174
	NCS-1	55
	NSF, SNX1	34
5-HT1A	Yif1B	31
	Calmodulin	192
5-HT1B	P11	22
5-HT1D	P11	22
5-HT2A	PSD-95	25
	β-arrestin 2 (Arrb2)	193
	Cav-1	86
	Calmodulin	194
	ARF-1	195
	MUPP1	196
	MAGI-2, SAP97, PSD-95, MPP3, CIPP, AOP-2	26
	RSK2	197
5-HT2B	MUPP1	196
5-HT2C	PSD-95, Veli3, CASK, Mint1, MPP3, Dynamin-1, calmodulin, PICOT, 4.1N, CAPZ β, CAPZ α-2, β-actin, α-fodrin, 2810409H07Rik protein	24
	PTEN	105
	β-arrestin 2 (Arrb2), calmodulin	64
	MUPP1	196
	MAGI-2, SAP97, SAP102, PSD-95, MPP3, Veli3	26
5-HT4	β-arrestin 1 (Arrb1), GPRK5	65
	P11	23
	MAGI-2, MPP3, Ulip2, SNX27, NHERF, guanine aminase, Veli1, Veli2, Veli3, peroxiredoxin 5, mNOS, CIPP, SEC23	39
5-HT6	Fyn kinase	198
	Jab1	199
5-HT7	PLAC-24/eIF3k	200
MT1	MUPP1	201

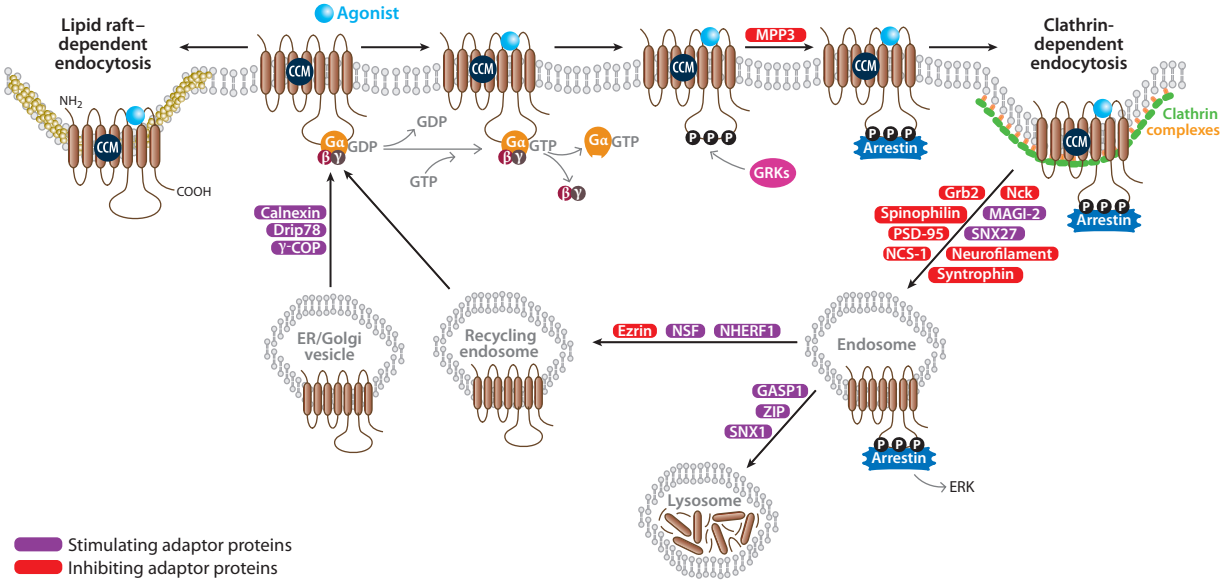

Figure 1

Schematic picture of how adaptor proteins and submembranous microdomains known as lipid rafts regulate monoamine receptor trafficking. Adaptor proteins can modulate monoamine receptor trafficking at several levels. (*Left*) The agonist (*blue sphere*) binding to the receptor leads to activation of G proteins and downstream effector molecules. The activated receptor is subsequently phosphorylated (*black circles*) by G protein–coupled receptor kinases (GRKs) (*magenta*), which promote arrestin (*dark blue*) recruitment. This results in desensitization of the receptor via uncoupling of the G protein and in clathrin-dependent endocytosis (*orange and green complexes*). The adaptor protein MPP3 counteracts desensitization of G protein–coupled receptors (GPCRs). MAGI-2 and SNX27 facilitate internalization of GPCRs, whereas Grb2, syntrophin, neurofilament-M, NCS-1, PSD-95, spinophilin, and Nck exert a negative influence on targeting of GPCRs to endosomes, some of them by anchoring the receptor to the plasma membrane. (*Right bottom*) Three routes are available for the internalized receptor: Either it is targeted for degradation, or it is sorted into recycling endosomes that bring the receptor back to the plasma membrane. Finally, internalization of the receptor in vesicles together with arrestin can facilitate G protein–independent signaling via extracellular regulated kinase (ERK). GASP1, SNX1, and ZIP target monoamine receptors for degradation, whereas NSF and NHERF1 promote recycling of receptors. Ezrin, on the other hand, has been shown to impair recycling. (*Left bottom*) Monoamine receptors are synthesized and maturate within the endoplasmic reticulum (ER)/Golgi vesicle; they then exit this subcellular compartment and are transported to the plasma membrane. Adaptor proteins are involved in several stages of this process. The adaptor proteins calnexin, Drip78, and γ-COP regulate transport of GPCRs from the ER/Golgi vesicle to the plasma membrane. Lipid rafts are known to mediate non-clathrin-dependent endocytosis of some monoamine receptors. In non-neuronal cells, this process often involves caveolae, but the mechanisms in neuronal cells are less understood. Adaptor proteins that stimulate the various processes are illustrated with purple rounded boxes, whereas those that exert negative influence are represented by red rounded boxes. Lipid-raft constituents are labeled as yellow dots in the plasma membrane. Abbreviation: CCM, cholesterol consensus motif (which binds cholesterol).

REGULATION OF MONOAMINE RECEPTOR TRAFFICKING BY ADAPTOR PROTEINS

A plethora of adaptor proteins have been found to modulate trafficking of monoamine receptors at several different stages, including maturation, internalization, targeting for lysosomal degradation, and recycling to the plasma membrane. As schematically illustrated in **Figure 1**, adaptor proteins can exert both positive and negative influences on such processes. A few examples illustrating important principles are reviewed here.

Regulation of Monoamine Receptor Signaling by β-Arrestins

The β-arrestins are critical regulators of GPCR signaling (6). These molecules bind to the receptor after agonist stimulation and subsequent phosphorylation by GRKs, and they facilitate desensitization and internalization of the receptor (6, 14). The two nonvisual isoforms of β-arrestin (1 and 2) share a high degree of sequence homology and are expressed in most mammalian cells, including neurons. They exhibit overlapping specificities and regulate numerous GPCRs (6). The arrestins were cloned in the early 1990s (15, 16) and identified as important regulators of β-adrenergic receptor (β-AR) signaling (6, 14, 17). Both β-arrestin 1 and 2 have also been shown to interact with additional monoamine receptors, including D2, D3, 5-HT2C, and 5-HT4 receptors (18–20). We do not further discuss the role of β-arrestin in trafficking and G protein–dependent signaling of GPCRs, as this topic has been reviewed extensively (6, 7).

The Adaptor Protein P11 Binds to 5-HT1B and 5-HT4 Receptors and Increases Their Surface Expression

P11 (also referred to as S100A10, calpactin 1 light chain, or annexin 2 light chain) is a member of the S100 family of Ca^{2+}-binding proteins (21). It is an inducible adaptor protein that binds to the 5-HT1B, 5-HT1D, and 5-HT4 receptors (22, 23). The distribution of P11 and these 5-HT receptors in the brain overlaps significantly, especially in the frontal cortex, ventromedial hypothalamus, hippocampus, and raphe nuclei, which are regions implicated in emotionality. Furthermore, the P11 protein colocalizes with the 5-HT1B and 5-HT4 receptors in the cell membrane of cultured cells. P11 binding augments the surface expression, the G protein–dependent signaling, and the extracellular regulated kinase (ERK) signaling exerted via the 5-HT1B and 5-HT4 receptors. In corticostriatal slices, P11 modulates serotonin-mediated depression of excitatory synaptic glutamatergic transmission, which is correlated with reduced phosphorylation of synapsin (22).

The Adaptor Proteins PSD-95 and Yif1B Regulate Surface Expression and Dendritic Targeting of Monoaminergic Receptors

The adaptor protein postsynaptic density 95 (PSD-95) is a member of a family of proteins referred to as PSD-95-disc-large-zonula occludens (PDZ) proteins. A common characteristic of this group of proteins is the PDZ motif, which enables scaffolding of signaling molecules. PSD-95 interacts with β_1-AR, 5-HT2A, and 5-HT2C receptors and regulates their function (24–27). Binding of PSD-95 to the β_1-AR decreases endocytosis of the receptor, whereas G protein–dependent signaling remains unaltered. Furthermore, binding of PSD-95 facilitates formation of a receptor complex that consists of β_1-AR and glutamate N-methyl-D-aspartic acid (NMDA)-type receptors (27). Similarly, PSD-95 binding inhibits internalization of the 5-HT2A receptor but has no effect on desensitization. In addition, PSD-95 recruitment results in augmented 5-HT2A signaling through the inositol phosphate pathway, possibly by scaffolding interactions between the receptor and signaling molecules such as phospholipase C. In addition to these effects, interaction between the 5-HT2A receptor and PSD-95 is crucial for targeting the receptor to dendrites in cortical neurons both in vitro and in vivo (28, 29). The effect of PSD-95 binding to the 5-HT2C receptor appears quite different from the effect of binding to β_1-AR or 5-HT2A receptors, as PSD-95 binding promotes both desensitization and agonist-induced internalization of 5-HT2C receptors (30). The mechanism underlying this differential response is not clear, but interactions between PSD-95 and the β_1-AR or 5-HT2A receptors may allow for recruitment of distinct sets of additional adaptor proteins, compared with the 5-HT2C receptor. It is possible that these distinct sets of protein complexes confer specificity in terms of different functionality of β_1-AR or 5-HT2A receptors versus the 5-HT2C receptor.

An additional example of dendritic targeting of a monoamine receptor by adaptor proteins is the interaction between Yip1 interacting factor homolog B (Yif1B) and the C-terminal region of the 5-HT1A receptor (31). This interaction is important for targeting the 5-HT1A receptors to distal parts of the dendrites. Further characterization of this interaction showed that these two proteins colocalize in intracellular small vesicles. The Yif1B protein is highly expressed in the brain, including in the raphe neurons, and it may have a role in trafficking vesicles between the Golgi apparatus and the endoplasmic reticulum (ER), implicating these systems in 5-HT1A receptor trafficking.

NHERF1, NSF, SNX1, and SNX27 Are Involved in Sorting Internalized Receptors for Recycling to the Plasma Membrane or Degradation in Lysosomes

Following agonist stimulation, most GPCRs are desensitized and internalized in an arrestin-clathrin-dependent manner and either recycled immediately to the cell membranes or directed to lysosomes for degradation. Several adaptor proteins influence the fate of internalized receptors. For example, the C-terminal tail of the β_2-AR receptor interacts with the adaptor protein Na^+/H^+ exchange regulatory factor 1 (NHERF1), which links the receptor to the actin cytoskeleton (32). This interaction is mediated via a PDZ motif and is dependent on phosphorylation of the receptor by GRK5. NHERF1 binding promotes recycling of the associated receptor, thus counteracting desensitization mechanisms (33). Interactions between dopaminergic receptors and adaptor proteins involved in receptor sorting have also been reported. Both D1 and D5 receptors have been found to bind N-ethylmaleimide-sensitive factor (NSF) (34), a protein that is also known to facilitate recycling of β_2-AR receptors to the plasma membrane (35). In the same study, sorting nexin 1 (SNX1), which targets some GPCRs for lysosomal degradation following endocytosis (36), was observed to interact specifically with the D5 receptor. Whether the aforementioned interactions with dopaminergic receptors have similar effects as those described for other receptors, however, remains to be elucidated. The C-terminal region of GPCRs provides binding sites for numerous adaptor proteins. The 5-HT4 receptor is arguably one of the GPCRs for which the largest number of splice variants has been described; this raises the possibility for multiple isoform-specific interactions (37, 38). Indeed, several adaptor proteins interact specifically with distinct 5-HT4 isoforms (39). The sorting nexin 27 (SNX27) protein is especially interesting as it interacts with 5-HT4(a) through its PDZ domain and facilitates trafficking of the receptor from the plasma membrane to early endosomes. However, it is not clear if SNX27 binding favors recycling of the associated receptor or degradation in lysosomes.

The Adaptor Proteins Drip78 and Calnexin Influence Transport and Maturation of Monoamine Receptors from the Endoplasmic Reticulum

GPCRs are synthesized and maturate within the ER/Golgi vesicle organelles before being transported to the plasma membrane. Adaptor proteins are involved at several stages of this process. The adaptor protein dopamine receptor-interacting protein of 78 kDa (Drip78) interacts specifically with the D1 receptor through an export sequence in the C-terminal region of the receptor (40). Modulation of cellular Drip78 levels results in retention of D1 receptors in the ER and reduced surface expression of the receptor. The study by Bermak et al. (40) points to an important role for adaptor proteins in regulating export of GPCRs from the ER. The observation that both D1 and D2 receptors bind to the adaptor protein calnexin lends further support to this idea. Using coimmunoprecipitation and mass spectrometry, Free and coworkers (41) identified a large number of potentially D1 receptor–interacting proteins. Interaction between the D1 and

also the D2 receptor with the ER chaperone protein calnexin was confirmed and further studied. The interaction with these receptors seems to be mediated via two distinct mechanisms—one that is glycan dependent and one that is not. The inhibition of calnexin resulted in accumulation of both subtypes of receptors in intracellular compartments. Significantly reduced surface expression levels of both receptors were also observed following overexpression of calnexin, probably caused by receptor retention in the ER. These data suggest that fine tuning of calnexin represents a regulatory mechanism for D1 and D2 receptor trafficking and surface expression.

REGULATION OF MONOAMINE G PROTEIN–DEPENDENT SIGNALING BY ADAPTOR PROTEINS

The classical paradigm of GPCR signaling posits that agonist binding to the receptor results in dissociation of the heterotrimeric G protein complex and in regulation of adenylate cyclase and second messengers such as cyclic adenosine monophosphate (cAMP) (**Figure 2**). GRKs subsequently phosphorylate the receptor, leading to recruitment of β-arrestin, which promotes desensitization and internalization of the receptor (6, 14). In addition to arrestins and GRKs, GPCRs interact with additional adaptor proteins that modulate their activity. Here we review how monoamine G protein–dependent signaling is regulated by adaptor proteins, exemplified by (*a*) GAIP-interacting protein, C terminus (GIPC); (*b*) spinophilin; and (*c*) Ca^{2+}-binding adaptor proteins. The involvement of these proteins in fine-tuning G protein–dependent D2 receptor signaling is shown in **Figure 2**.

D2 and D3 Receptor Signaling Is Regulated by the Adaptor Proteins GIPC and GAIP

The D2 and D3 receptors, but not the D4 receptors, interact via their C termini with the adaptor protein GIPC both in vitro and in vivo (42). The interaction appears to be mediated through PDZ and acyl carrier protein (ACP) recognition motifs at the C terminus of the adaptor protein. When coexpressed in cell lines, both D2 and D3 receptors induce translocation of GIPC to the cell membrane. GIPC cointernalizes with both receptors, which may suggest a role for this adaptor protein in endocytic vesicle sorting. In addition, GIPC appears to be a negative regulator for signaling via both D2 and D3 receptors. Regarding the D2 receptor, GIPC recruitment enables the formation of a complex consisting of the receptor, GIPC, and GAIP (also referred to as RGS19) (43). GAIP belongs to the regulator of G protein signaling (RGS) proteins, which are inhibitors of G protein–dependent signaling. They exert their function by directly interacting with activated G proteins and facilitating hydrolysis of bound GTP to GDP, which terminates signaling (44). Following agonist stimulation, GAIP rapidly translocates to the cell membrane in a GIPC-dependent manner, where it inhibits D2 receptor signaling. A similar reduction in signaling has been reported for the D3 receptor in the presence of GAIP. However, whether this effect can be attributed to a similar mechanism remains to be determined. Furthermore, GIPC shows overlapping mRNA distribution patterns with that of the D2 receptor in hippocampus and striatum and with that of the D3 receptor in the islands of Calleja of the rat brain, further suggesting a potential role for this interaction in vivo.

Spinophilin Modulates G Protein–Dependent Ca^{2+} and MEK Signaling of the α-Adrenergic Receptor

Spinophilin (also referred to as neurabin II) is a binding partner to the catalytic subunit of protein phosphatase 1 (PP1) that robustly attenuates PP1 enzymatic activity (45). Further analysis revealed

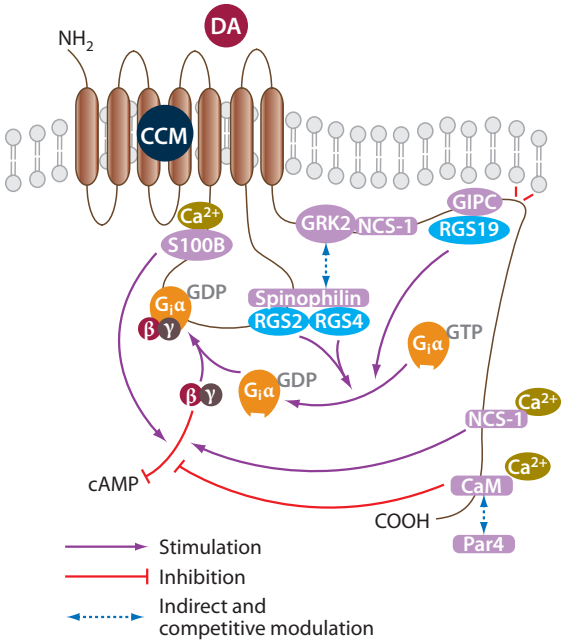

Figure 2

Regulation of D2 receptor G protein–dependent signaling by adaptor proteins. The D2 receptor contains a cholesterol consensus motif (CCM; *dark blue dot*) between helices I, II, III, and IV, which indicates that it is modulated by direct interactions with cholesterol. The D2 receptor is negatively coupled to adenylate cyclase via G_i, which binds to the third intracellular loop. Dopamine (DA; *dark red dot*) binding to the D2 receptor results in exchange of bound GDP for GTP and dissociation of the heterotrimeric G protein complex, resulting in inhibition of adenylate cyclase and reduced levels of cyclic adenosine monophosphate (cAMP). The Ca^{2+} (*dark yellow dot*)-binding protein S100 protein beta polypeptide (S100B) interacts with the third intracellular loop of the receptor and augments G protein–dependent signaling via the D2 receptor. Similarly, the Ca^{2+}-binding protein NCS-1 (neuronal calcium sensor 1) binds to the C-terminal portion of the receptor and enhances D2 receptor–mediated inhibition of cAMP. Adaptor proteins (*light violet*) can provide a mechanism for receptor recognition for molecules involved in termination of G protein–dependent signaling, as can be exemplified by spinophilin and GAIP-interacting protein, C terminus (GIPC). Spinophilin binds to the third intracellular loop of the receptor and recruits RGS2 and RGS4 proteins (*light blue*); GIPC binds to the C termini and scaffolds RGS19 (also referred to as GAIP). The main function of regulator of G protein signaling (RGS) proteins is to facilitate hydrolysis of receptor-bound GTP to GDP, thereby terminating G protein–dependent receptor signaling. Competitive binding between spinophilin and GRK2 (*light violet*) regulates β-arrestin recruitment and G protein–dependent MAPK/ERK kinase 1/2 (MEK) signaling. The adaptor protein prostate apoptosis response 4 (Par4) interacts with C termini of the D2 receptor and facilitates inhibition of cAMP and downstream effectors such as cAMP response element binding protein (CREB). Calmodulin (CaM) can compete with Par4 binding via a Ca^{2+}-dependent mechanism and inhibit D2 receptor signaling. Stimulation is indicated by purple arrows; inhibition, by red arrows. Indirect and competitive modulation is indicated by dashed blue lines. Indirect modulation occurs between GRK2 and spinophilin, and between Par4 and CaM.

that this protein also binds to actin (46). Spinophilin is highly enriched in dendritic spines and is important for their formation and regulation (47, 48). Spinophilin is expressed predominantly in the hippocampal formation, and to a lesser extent in the caudatoputamen and dorsal thalamus of the rat brain. Expression levels of spinophilin increase during development of the brain, pointing to a potential neurodevelopmental role, and then subside even though it remains expressed in the adult rat brain. Spinophilin binds to the third intracellular loop of several GPCRs, including

adrenergic and dopaminergic receptors (49, 50), and regulates their signaling (51, 52). Binding of this adaptor protein to the third intracellular loop of the α_{2B}-AR attenuates internalization of this receptor, and consequently, turnover is dramatically increased in cells that lack the spinophilin gene (52). Spinophilin has been found to bind to the same region of the D2 receptor, but the functional consequences of this interaction are unclear (49). In addition to GPCRs, spinophilin interacts with RGS proteins, specifically RGS1, RGS2, RGS4, RGS16, and GAIP. Wang and coworkers showed that spinophilin forms a signaling complex with the α-AR and RGS2 (50). This interaction enhances RGS2-mediated inhibition of α-AR Ca^{2+} signaling. Thus scaffolding by spinophilin seems to provide a mechanism for receptor recognition and recruitment of the RGS proteins near the receptor to terminate G protein–dependent signaling.

β-Arrestins and spinophilin induce opposing effects on receptor internalization, so it is plausible to deduce that these molecules competitively regulate receptor trafficking and signaling. Such a mechanism has indeed been reported for the α_2-AR (53). Binding of spinophilin in favor of GRK2 results in decreased phosphorylation and attenuated β-arrestin-mediated internalization of the α_2-AR. Spinophilin also seems to attenuate desensitization of G protein–dependent MAPK/ERK kinase 1/2 (MEK) signaling in vivo, whereas β-arrestin 2 has the converse effect. Opposing regulation by spinophilin and β-arrestin also influences the sedative response to α-AR agonists in vivo—i.e., mice lacking the spinophilin gene were more sensitive to their sedative properties—whereas β-arrestin knockout mice were more resistant. The observation described above might represent a general mechanism of receptor regulation, and there is some evidence that it also applies to the Na^+K^+-ATPase (54). However, there are no data on additional GPCRs regulated by this mechanism.

Regulation of D2 Receptor Signaling by the Ca^{2+}-Binding Proteins NCS-1 and S100B

The neuronal calcium sensor 1 (NCS-1) protein is a member of the EF-hand Ca^{2+}-binding protein family, which is known to exert a range of different functions regarding neuronal signaling (11). Interaction with the adaptor protein NCS-1 at the C terminus of D2 receptors facilitates the formation of a signaling complex consisting of the D2 receptor, NCS-1, and GRK2 (55). Binding of NCS-1 to the receptor counteracts GRK-dependent internalization of the receptor, whereas it augments G protein–dependent signaling. Moreover, Ca^{2+} binding by NCS-1 seems to be a prerequisite for both these effects. Remarkably, the D2/NCS-1 interaction may also play a role in the expression of complex behavioral phenotypes in vivo (56). Selective overexpression of NCS-1 in the dentate gyrus of mice results in increased exploratory behavior, facilitation of long-term potentiation (LTP) in the medial perforant pathway, and enhancement of rapid-acquisition spatial memory. These effects could be counteracted through D2 receptor antagonists or a small interfering peptide that disrupts interaction between the receptor and NCS-1. These studies (55, 56) highlight the importance of NCS-1 in D2 receptor regulation.

S100 protein beta polypeptide (S100B) is another EF-hand Ca^{2+}-binding protein, which belongs to the same family as S100A10 (i.e., P11), a 5-HT1B/4 receptor adaptor protein (see above). S100B interacts with the D2 receptor (57). This protein is expressed at high levels in the brain, predominantly in astrocytes, but also to a lesser extent in neural progenitor cells and neurons (58, 59). S100B expression is highly inducible and has been shown to be regulated by a number of different stimuli, including growth factors, immunological mediators, and toxic insults. The S100B protein exerts a positive influence on cell proliferation and migration but acts as an inhibitor on differentiation and apoptosis (58). Furthermore, S100B may serve as a potential biomarker for neurodegeneration (58). In the CNS, this protein has been reported to be implicated in axonal

G PROTEIN–INDEPENDENT SIGNALING OF GPCRS

Several GPCRs can signal in a G protein–independent manner. Such signaling has been reported for monoamine receptors. Studies on the β-arrestins suggest that adaptor proteins play an important role in conveying G protein–independent signaling. During this process, the arrestins link the GPCR to a multitude of intracellular mediators, which, in turn, can modulate the activity of key signaling molecules.

proliferation and neurite outgrowth (60, 61). S100B colocalizes and interacts with the D2 receptor via a binding motif within the third intracellular loop (57). This motif is not present in the D3 receptor, and, accordingly, this receptor does not appear to interact with the adaptor protein. Binding of S100B is associated with increased ERK signaling and inhibition of adenylate cyclase by the D2 receptor. Whether these effects are dependent on Ca^{2+} binding, however, has not been determined.

REGULATION OF MONOAMINE G PROTEIN–INDEPENDENT SIGNALING BY ADAPTOR PROTEINS

G protein–independent signaling has been reported for several monoamine receptors, including β_2-AR, D2, 5-HT2C, and 5-HT4 receptors (62–65) (see sidebar, G Protein–Independent Signaling of GPCRs). Studies on β-arrestin suggest that adaptor proteins play an important role in conveying such signaling (6, 66). However, it is likely that the β-arrestins are not the only adaptor proteins that can convey this type of signaling. During G protein–independent signaling, the arrestins link the GPCR to a multitude of intracellular mediators. The signaling complex that is then formed can modulate the activity of key signaling molecules such as v-akt murine thymoma viral oncogene homolog 1 (AKT) and ERK (6, 62, 67). G protein–independent interactions between adaptor proteins and a D2 receptor are shown in **Figure 3**.

Arrestin-Mediated G Protein–Independent Signaling by the β_2-Adrenergic Receptor

The regulation of β_2-AR desensitization and internalization has been studied extensively (6, 14, 17). Following agonist stimulation, arrestins are recruited to the receptor, which facilitates uncoupling of the G_s protein and increased clathrin-mediated internalization (6). Furthermore, the β_2-AR can activate ERK through both G protein–dependent and –independent pathways (68). The G protein–dependent pathway is characterized by a transient activation pattern with rapid onset and termination, whereas the G protein–independent pathways show much slower onset but are sustained for a significantly longer time period. The latter pathway is dependent on β-arrestin, which appears to form a transient complex with the receptor and ERK (69). Interestingly, this complex is stabilized by coexpression of GRK5 or GRK6, leading to accumulation of β-arrestin complexes in endosomes, which also is associated with a robust increase in ERK phosphorylation. Thus it seems likely that the β_2-AR can facilitate G protein–independent, arrestin-dependent ERK signaling in an endosomal manner similar to that previously described for the angiotensin II (ATI) receptor (67), which differs in both cellular distribution and temporal aspects compared with G protein–dependent signaling (63, 70).

β-Arrestin-Mediated Signaling by the D2 Receptor

In addition to the classical inhibitory G protein–dependent signaling, the D2 receptor has been shown to signal through a β-arrestin-dependent pathway (62). Agonist stimulation of the D2

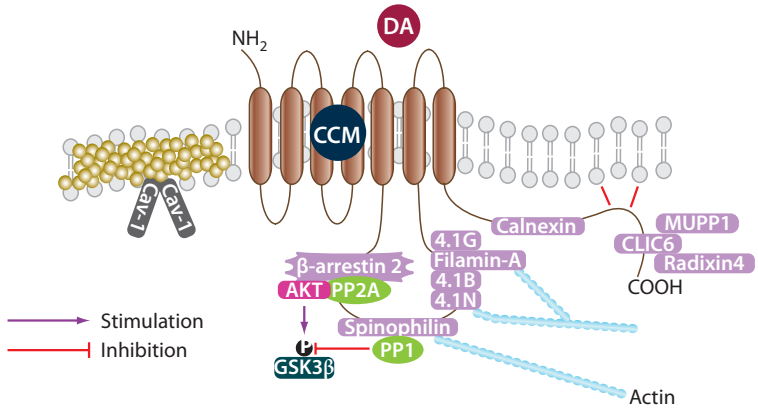

Figure 3

Schematic picture of how adaptor proteins regulate G protein–independent signaling and trafficking via D2 receptors. Stimulation of the D2 receptor by dopamine (DA; *dark red dot*) leads to recruitment and binding of the adaptor protein β-arrestin 2 (*light violet*) to the third intracellular loop of the receptor and promotes the formation of a signaling complex consisting of the receptor, β-arrestin 2, protein phosphatase 2A (PP2A; *light green*), and v-akt murine thymoma viral oncogene homolog 1 (AKT; *magenta*). After PP2A is brought within proximity of AKT, it can dephosphorylate AKT. This leads to decreased efficiency of this enzyme, and, as a consequence, disinhibition of glycogen synthase kinase 3β (GSK3β), whose activity is upregulated. Adaptor proteins (*light violet*) can interact with the D2 receptor. Spinophilin binds to the third intracellular loop of the D2 receptor and scaffolds protein phosphatase 1 (PP1; *light green*), which inhibits GSK3β. Several actin-binding proteins—including 4.1N, 4.1B, 4.1G, and Filamin-A—bind to the same region of the receptor and to actin (*light blue rods*), which links the receptor to the cytoskeleton. Heart type fatty acid binding protein (H-FABP) binds to the receptor and presumably links the receptor to the plasma membrane through its lipid-binding domains. Calnexin and the CLIC6 complex interact with the C termini with unknown functional consequences. Caveolin-1 (cav-1) has been shown to promote internalization of D2 receptors via a largely unknown mechanism. CCM, cholesterol consensus motif.

receptor leads to a recruitment of β-arrestin 2 to the receptor at the cell membrane. Acting as a scaffold, β-arrestin 2 promotes the formation of a signaling complex that consists of the receptor, β-arrestin 2, protein phosphatase 2A (PP2A), and the protein kinase AKT. Assembly of this complex results in a marked and sustained dephosphorylation of AKT at an important regulatory site (Thr-308). Dephosphorylation at this site reduces the ability of AKT to suppress the downstream target glycogen synthase kinase 3β (GSK3β), whose activity is upregulated as a consequence. The cellular presence of β-arrestin 2 also appears to be a prerequisite for complex formation because interaction between AKT and PP2A is abolished in mice lacking this gene. Furthermore, both dephosphorylation of AKT and psychostimulant-induced locomotion are strongly attenuated in such mice, indicating that G protein–independent, arrestin-dependent AKT signaling is crucial for behavioral manifestations induced by altered dopaminergic neurotransmission (see below).

G Protein–Independent Signaling by 5-HT Receptors Is Both Positively and Negatively Influenced by β-Arrestin

Both 5-HT2C and 5-HT4 receptors are capable of G protein–independent signaling (64, 65). Stimulation by 5-HT recruits β-arrestin 2 and calmodulin to the 5-HT2C receptor, and this interaction appears to be essential for G protein–independent ERK signaling (64). Similarly, stimulation of the 5-HT4 receptor results in G protein–independent activation of ERK through

CHOLESTEROL CONSENSUS MOTIF

Recent crystallization studies on adrenergic receptors provide structural evidence that the receptor binds to cholesterol via a cholesterol consensus motif (CCM). The motif, which is four amino acids long, is present in the majority of the 5-HT receptors as well as other monoamine receptors. These findings are in concordance with earlier studies, which suggests that GPCR signaling is directly modulated by cholesterol binding.

a mechanism that involves the tyrosine kinase Src. Notably, this type of signaling is inhibited by GRK5 and β-arrestin 1 binding to the receptor (65). Two conclusions can be drawn from these studies: (*a*) β-arrestin regulation of G protein–independent signaling is complex, and (*b*) it exerts both activating and inhibitory effects. Furthermore, it is plausible that recruitment of Src to 5-HT4 receptors and subsequent ERK activation are aided by hitherto unknown interacting proteins.

CHOLESTEROL, LIPID RAFTS, AND CAVEOLINS REGULATE MONOAMINE RECEPTOR FUNCTION

Monoamine receptors are regulated not only by protein-protein interactions but also by protein-lipid interactions. The subcellular localization and signaling properties of monoamine receptors are affected by the lipid microenvironment. In particular, two recent lines of research have pointed to the importance of lipids in regulating monoamine receptor pharmacology: signaling and trafficking. First, the recently published crystal structure of the β_2-AR provides evidence that the receptor binds to cholesterol via a cholesterol consensus motif (CCM) that is four amino acids long (71, 72) (see sidebar, Cholesterol Consensus Motif). In accordance with previous observations that cholesterol affects binding of ligands to at least some 5-HT receptors (73), the CCM is also present in the majority of the 5-HT receptors as well as other monoamine receptors. Second, the discovery of lipid rafts and other membranous microdomains has caused a reassessment of the classic Singer-Nicolson fluidic mosaic model, which described cell membranes as, more or less, homogeneous phospholipid bilayers (74). It is now clear that the plasma membrane is heterogeneous and plays a far greater part in cellular functions than merely serving as a barrier and anchor for proteins.

Compared with other compartments of the plasma membrane, lipid rafts are highly ordered structures that are rich in saturated lipids, such as cholesterol, sphingomyelin, glycosphingolipids, gangliosides (GM1 and GM3), and ceramides (75, 76) (see sidebar, Lipid Rafts). The functions of lipid rafts include clustering of receptors with proteins involved in signal transduction, such as various G proteins and adenylate cyclase subtypes (77, 78). In addition to this role, lipid rafts also facilitate non-clathrin-mediated internalization of GPCRs (**Figure 1**) (75, 79). A subgroup

LIPID RAFTS

Lipid rafts are highly ordered structures within the plasma membrane that are rich in saturated lipids and scaffolding proteins. The functions of lipid rafts include clustering of receptors with proteins involved in signal transduction, such as various G proteins and adenylate cyclase subtypes. In addition to this role, lipid rafts also facilitate non-clathrin-mediated internalization of GPCRs.

CAVEOLAE

Caveolae is a subgroup of lipid rafts that contains caveolin and cavin proteins. Caveolae are present in many cell types, but neurons exhibit no functional caveolae. Despite the lack of caveolae, some populations of neurons do in fact express cav-1 proteins, which seem to play a functional role in modulating neuronal plasticity and neurite outgrowth.

of lipid rafts referred to as caveolae contain caveolin proteins (cav-1–3) as well as cavin proteins (cavin 1–4) (80) (see sidebar, Caveolae). Caveolae are present in many cell types, but neurons exhibit no functional caveolae (81). Most studies on caveolae and lipid rafts have been performed in non-neuronal cells. However, despite the lack of caveolae, some populations of neurons do in fact express cav-1 proteins, which seem to play a functional role in modulating neuronal plasticity and neurite outgrowth (82, 83). In fact, cav-1 knockout mice exhibit deficits in emotionality, cognition, and locomotion, including increased anxiety, spatial memory impairments, reduced motor activity, and gait abnormalities (84, 85).

There is evidence that caveolins interact directly with some monoamine receptors. Cav-1 binds to the 5-HT2A receptors in an interaction that seems to be a prerequisite for recruitment of G_q proteins to the receptor (86), whereas both cav-1 and cav-2 have been found to coimmunoprecipitate and functionally interact with D1 receptors (87, 88). β_2-ARs have been shown to directly interact with cav-1 and to be enriched in caveolae in heterologous cell lines (89). Likewise, studies in myocytes have found that β_2-ARs are localized to caveolae, where they interact with cav-3 (90). More recently, immunoprecipitation studies in myocytes have indeed demonstrated that cav-3, Ca(v)1.2, β_2-AR (but not β_1-AR), G_s, adenylate cyclase, protein kinase A (PKA), and PP2A form a macromolecular signaling complex that is specifically required for proper β_2-AR signaling in myocytes (91). Several studies have also shown that monoamine receptors colocalize and/or comigrate with caveolins in submembranous lipid-raft fractions. Cav-1 has been shown to be localized in close proximity to D2 receptors and to promote their internalization and heterodimerization with adenosine A2A receptors (92). α_{2A}-ARs, but not α_{2B}-ARs, are internalized via a caveolin-dependent mechanism (93). Cav-1 and 5-HT7 receptors are colocalized in lipid rafts, and siRNA-mediated knockdown of cav-1 was shown to attenuate agonist binding at 5-HT7 receptors (94). 5-HT1A receptors, together with cav-1, have been shown to distribute to lipid-raft fractions in a palmitoylation-dependent manner (95).

Monoamine receptors are also regulated by sequestration and/or synthesis inhibition of cholesterol, sphingomyelin, and/or gangliosides. Reduction of either of these lipid-raft constituents reduced ligand binding to the 5-HT7 receptor (96, 97). Cholesterol sequestration with methyl-β-cyclodextrin modulates both signaling and ligand binding to 5-HT1A receptors (73, 98). Moreover, specific ligand binding to 5-HT1A receptors is increased upon sphingomyelinase treatment (99). Several lines of evidence support the idea that caveolins and lipid rafts modulate monoamine receptors, but there are still limited data on such modulation in neuronal cells.

ADAPTOR PROTEINS AND LIPID RAFTS IN DISEASE STATES AND EFFECTS OF DRUGS AND SOME THERAPEUTIC AGENTS

A clear-cut example of how an adaptor protein can be involved in a disease state is provided by a protein that modulates the melanocortin 2 receptor (MC2R). Mutations in an adaptor protein termed MC2 receptor accessory protein (MRAP) have been found to be associated with familial

LIGAND BIAS

This concept adheres to the observations that different ligands of the same receptor can elicit a differential response in terms of adaptor protein recruitment, receptor trafficking, G protein–dependent signaling, and G protein–independent signaling that is specific to each ligand. This concept may have potential use in the development of new pharmacotherapies.

glucocorticoid deficiency, an autosomal recessive disorder resulting from resistance to the action of adrenocorticotropic hormone (ACTH) on the adrenal cortex (100). MRAP may have a role in trafficking the MC2R from the endoplasmic reticulum to the cell surface. Regarding adaptor proteins that modulate monoaminergic receptor signaling, the association is less direct. Nonetheless, several such proteins may be involved in a number of disease states and mediate the effect of pharmacotherapies. Alterations of constituents of lipid rafts, including cholesterol, gangliosides, and sphingomyelin, are known to cause disease states such as Smith-Lemli-Opitz syndrome, Niemann-Pick C, and GM1 and GM2 gangliosidoses with diverse neurological dysfunctions that have been reviewed elsewhere (101–103). Likewise, several central actions, particularly related to neurodegeneration, of orally administered statins and other cholesterol-modifying agents have been summarized elsewhere (104) and are not discussed here. We discuss how adaptor proteins and lipid rafts are implicated in psychiatric and neurological disorders according to the following criteria: (*a*) how levels of adaptor proteins and lipid rafts are involved in the pathogenesis of psychiatric disorders, (*b*) how adaptor proteins or lipid rafts affect drug availability and efficacy, (*c*) ligand bias and its implication for pharmacotherapy (see sidebar, Ligand Bias), and (*d*) the potential use of interfering peptides in pharmacotherapy (see sidebar, Interfering Peptides). G protein–independent and –dependent actions of existing or experimental agents directed toward D2 receptors are illustrated in **Figure 4**. A few general comments on interfering peptides and biased ligands, however, precede the more detailed discussion on the role of adaptor proteins and lipid rafts in disease states, drugs, and therapeutic agents.

Targeting Adaptor Proteins: Interfering Peptides and the Concept of Biased Ligands, and Implications for Pharmacotherapy

Over the past years, two parallel strategies have emerged for modifying GPCR signaling by targeting adaptor protein–receptor interactions. The first one is targeting receptor complexes through the use of interfering peptides, which competitively block protein-protein interactions. Recent studies on D2 and 5-HT2C receptors (56, 105) have demonstrated the feasibility of this concept in rodents in vivo (see below). Interfering peptides have also been used to modulate NMDA receptor signaling (106). This strategy may have widespread applications and could provide a

INTERFERING PEPTIDES

Interfering peptides are devised to interfere with adaptor protein–receptor complexes. These peptides are fused to recognition sequences, which allow them to penetrate the cell surface and competitively block protein-protein interactions. This strategy has been used successfully to disrupt adaptor protein interactions and modulate D2 and 5-HT2C receptor signaling in rodents in vivo.

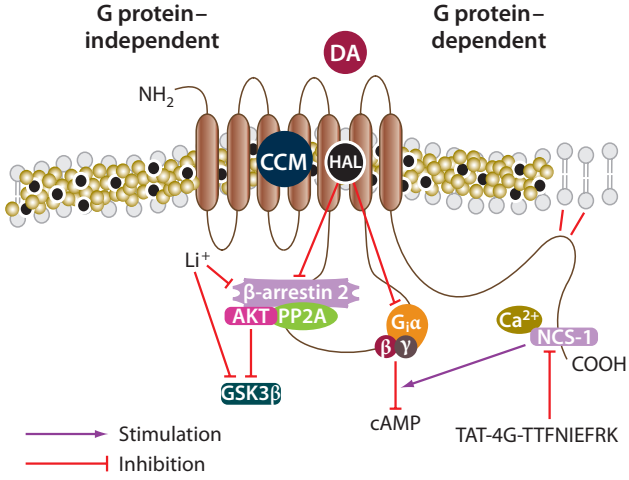

Figure 4

Targeting adaptor proteins and lipid rafts affects response and distribution of pharmacological agents at the D2 receptor. Antipsychotics such as haloperidol (HAL; *black dots*) bind the receptor and antagonize signaling through both classical G protein–dependent pathways (*right*) and arrestin-dependent, G protein–independent AKT signaling (*left*). The mood stabilizer lithium inhibits GSK3 β (*dark aqua*) at two levels, first by disrupting the signaling complex that consists of β-arrestin 2 (*light violet*), PP2A (*light green*), and AKT (*magenta*) and then by direct inhibition of GSK3 β. Ca^{2+}-dependent binding of NCS-1 (*light violet*) to the C terminus of the receptor counteracts internalization and augments G protein–dependent signaling of the receptor. Interfering cell-penetrating peptides can be used to disrupt NCS-1 binding to the D2 receptor, affecting both molecular and behavioral aspects of dopaminergic signaling. Several dopaminergic receptors are located in lipid-raft microdomains (*yellow lipid moieties*). Antipsychotics (*small black dots*), including haloperidol, have been shown to accumulate in lipid rafts and disrupt lipid-raft composition, distribution of G proteins, and GPCR signaling. Purple arrows indicate stimulation; red arrows indicate inhibition. Abbreviations: AKT, v-akt murine thymoma viral oncogene homolog 1; cAMP, cyclic adenosine monophosphate; CCM, cholesterol consensus motif; DA, dopamine; GPCR, G protein–coupled receptor; GSK3, glycogen synthase kinase 3; NCS-1, neuronal calcium sensor 1; PP2A, protein phosphatase 2A.

more specific means to modify monoamine receptor signaling compared with traditional agonists or antagonists. Considering that the potential use of interfering peptides in pharmacotherapy has recently been extensively reviewed (107), we do not further discuss the topic in this review. The second strategy stems from the realization that GPCRs can signal through both G protein–dependent and –independent pathways. This discovery represents a paradigm shift, and several theoretical frameworks have been proposed to incorporate these ideas into the overall theory on GPCR signaling, including ligand bias, biased agonism, functional selectivity, and ligand-directed trafficking or signaling (66, 108, 109). In this review, we use the term ligand bias. This concept adheres to the observations that different ligands of the same receptor can elicit a differential response in terms of adaptor protein recruitment, receptor trafficking, G protein–dependent signaling, and G protein–independent signaling that is specific to each ligand. Studies on β-arrestin suggest that recruitment of adaptor proteins to GPCRs plays an important role in determining such specificity (66). The task of assessing pharmacological efficacy now demands taking both G protein–dependent and –independent signaling into consideration, where each pharmacological agent will display specific agonistic or antagonistic profiles for each type of signaling (66). Development of ligands that are completely selective for one type of signaling—i.e., perfectly biased ligands—would greatly improve the knowledge of how G protein–dependent signaling and –independent signaling mediate different aspects of monoamine receptor signaling. This improved

knowledge could have potential clinical applications. Another approach for this purpose may involve studies of currently used monoaminergic pharmacotherapies and correlate physiological responses with the agonistic or antagonistic properties of these pharmacotherapies on G protein–dependent versus –independent pathways (see Reference 110). Finally, biased agonists are best characterized for β-arrestin-dependent, G protein–dependent signaling, but other adaptor proteins can probably facilitate such signaling as well. Consequently, the complexity of assessing pharmacological efficacy is likely to grow as additional adaptor proteins that mediate G protein–independent signaling are identified.

Addiction and Actions of Drugs of Abuse

A cardinal feature of addictive disorders is the loss of control of drug intake. This is accompanied by other symptoms related to both physiological and behavioral aspects of the disorder, as described, for example, in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV). In light of the fact that all drugs of abuse activate the mesolimbic dopamine system, a long line of researchers have investigated the role of this system in the development of addiction (111–113). Recent literature, however, suggests that additional systems are important, notably glutamatergic and corticotropin-releasing hormone (CRH) signaling (114–116). Currently used pharmacotherapies for addiction include naltrexone, a μ opioid receptor antagonist; acamprosate, which presumably antagonizes NMDA receptors (117); and methadone, a slow-acting agonist of the opioid receptors (118).

The drug 3,4-methylenedioxy-metamphetamine (MDMA, also referred to as ecstasy) has been shown to augment 5-HT and dopaminergic neurotransmission. Chronic MDMA treatment was found to increase gene expression of S100B (119). The functional impact of increased S100B expression with respect to cellular and behavioral parameters was assessed through the use of transgenic mice that overexpressed S100B. MDMA binges decreased measures of anxiety-like behavior to a higher extent in S100B in some paradigms compared with wild-type mice. This correlated with genotype-specific alterations in basal 5-HT transporter expression and MDMA-induced 5-HT1B receptor binding and activity, but not in MDMA-induced downregulation of dopamine transporter expression. These data indicate that genetic differences in S100B gene expression may underlie individual differences in the responsivity to chronic intake of MDMA. Some of the effects of S100B levels on the response to MDMA could be mediated through the D2 receptors because, as previously described, S100B binds to this receptor and augments its signaling (57).

Several aspects of β-arrestin 2 regulation of GPCR signaling suggest that this molecule is involved in mediating the rewarding effects of drugs of abuse and the development of drug addiction. The first evidence supporting this notion was the observation that β-arrestin 2 is a critical regulator of μ opioid receptor desensitization in vivo (120). Activation of μ opioid receptors on γ-aminobutyric acid (GABA)-ergic interneurons in the ventral tegmental area (VTA) and facilitation of subsequent accumbal dopamine release are important components for the rewarding effects of many drugs of abuse (113, 121). Consistent with the fact that β-arrestin 2 knockout mice display potentiated and prolonged μ opioid receptor signaling, they also display increased dopamine release in the nucleus accumbens and experience greater reward in response to morphine, a prototypical μ opioid receptor agonist (122). These data suggest that β-arrestin 2 is a negative regulator of morphine reward. In contrast, the opposite relationship seems to apply to the rewarding properties of amphetamine and ethanol (62, 123) because β-arrestin 2 knockout mice display reduced locomotor behavior in response to both drugs, often regarded as a proxy measurement of drugs' rewarding effects. For ethanol reward, this idea is further supported by

observations that ethanol-evoked dopamine release appears unaltered (K. Björk, G. Tanda, A. Thorsell, M. Heilig, and W.H. Sommer, unpublished results), whereas c-fos activation in the shell of the nucleus accumbens is abolished in β-arrestin 2 knockout mice following a stimulatory dose of ethanol (123). Together, these studies suggest that β-arrestin 2 can modulate drug reward at two levels: first, as in the case of morphine, by modulating opioid receptor signaling in the VTA and subsequently disinhibiting mesolimbic dopaminergic signaling; and second, as for amphetamine and ethanol, by mediating postsynaptic dopamine receptor signaling in the nucleus accumbens.

The adaptor protein PSD-95 is a critical modulator of dopaminergic and serotonergic signaling in response to psychostimulants and hallucinogenic drugs, respectively. Using high-throughput global expression profiling, Yao et al. (124) identified *PSD-95* as a gene that is downregulated in the striatum in hyperdopaminergic states, induced either by genetic manipulation or as an effect of chronic cocaine treatment. The functional impact of this finding was assessed using transgenic mice lacking striatal PSD-95. These mice exhibited exaggerated responses to acute treatment with cocaine but failed to develop locomotor sensitization following chronic cocaine administration. This study (124) demonstrates a critical role for PSD-95 in both molecular and behavioral plasticity associated with chronic cocaine exposure.

Several reports demonstrate that the hallucinogenic properties of 5-HT2A receptor agonists are dependent on ligand and highly affected by interactions with adaptor proteins. The hallucinogenic actions of the drug 2,5-dimethoxy-4-iodoamphetamine (DOI) are dependent on PSD-95 modulation of the 5-HT2A receptor. Abolishment of PSD-95 in vivo resulted in strongly attenuated head-twitch response, a measure of hallucinogenic properties of a drug in mice, and increased receptor internalization and impairments in 5-HT2A receptor–mediated intracellular signaling (29). However, different 5-HT2A agonists seem to be dependent on different adaptor proteins to induce hallucinogenic responses. Schmid and coworkers (125) showed that treatment with either 5-HTP (a precursor of 5-HT) or DOI induces head twitches. However, for 5-HTP, head-twitch response as well as agonist-induced internalization of the 5-HT2A receptor and subsequent ERK signaling is dependent on β-arrestin 2, whereas treatment with DOI elicits these processes in the absence of this adaptor protein (125). Paralleling these studies, results from Gonzalez-Maeso et al. (126) showed that nonhallucinogenic and hallucinogenic agonists of the 5-HT2A receptors activate distinct intracellular signaling pathways and gene expression profiles. Taken together, these results strongly suggest that the overall response to different 5-HT2A agonists is ligand specific and crucially dependent on the selective recruitment of adaptor proteins. The adaptor proteins, in turn, determine receptor-mediated effects on trafficking, signaling, and transcriptional regulation, as well as behavioral responses.

The 5-HT system inhibits the mesolimbic dopamine system via activation of 5-HT2C receptors located on dopaminergic neurons in the VTA. This receptor has been implicated in addiction-related phenotypes such as sensitization, place preference, and self administration (127) and is targeted by atypical antidepressants, presumably reversing a hypodopaminergic state associated with the disorder (10). A study by Ji and coworkers (105) showed that the adaptor protein phosphatase and tensin homolog deleted on chromosome 10 (PTEN) binds to the third intracellular loop of the 5-HT2C receptor, both in vitro and in vivo, and that it counteracts agonist-induced phosphorylation of the receptor. By administration of a peptide that interferes with formation of the 5-HT2C-PTEN complex in vivo, they were able to show that PTEN binding exerts an inhibitory effect on 5-HT2C receptor signaling and consequently leads to activation of mesolimbic dopaminergic neurons. Pretreatment with the interfering peptide blocked the ability of nicotine and tetrahydrocannabinol to augment firing rates of VTA dopamine neurons and to promote conditioned place preference, a measure of a drug's rewarding properties. Similar

results could be obtained for both measures using a specific 5-HT2C receptor agonist, but in contrast to the peptide, the 5-HT2C agonist induced a range of other effects such as increased anxiety, penile erection, hypophagia, and motor functional suppression. This study (105) shows that PTEN interacts with the 5-HT2C receptor and that this interaction is crucial for the rewarding effects of drugs of abuse. In addition, it suggests that the interfering peptide might offer a novel strategy to treat drug addiction with fewer side effects. However, even though the peptide strategy in itself is interesting and might have widespread applications, it should be noted that modulation of dopaminergic signaling has achieved limited success in the treatment of addiction (128, 129).

Schizophrenia and the Mechanism of Action of Antipsychotics

Schizophrenia is a psychiatric disorder characterized by positive and negative symptoms. The positive symptoms may include delusions, hallucinations, and disordered speech and thinking. Negative symptoms are associated poor emotional expression, social withdrawal, and lack of motivation and interest in activities normally considered pleasurable. A third set of cognitive symptoms may be present before the onset of the disorder. Such symptoms include attention deficits, impaired working memory and verbal fluency, and dysregulated executive functions important for organizing and planning. Also, at the neurochemical level, schizophrenia is a multifaceted disease with changes in glutamate, GABA, dopamine, and serotonin transmission. All antipsychotics are D2 receptor antagonists, some of which also antagonize the 5-HT2A receptors.

A few studies have investigated the association between adaptor protein levels and psychiatric disorders in human tissue. Both transcript and protein levels of the Ca^{2+}-binding proteins NCS-1 and calcyon have been found to be upregulated in the prefrontal cortex of deceased patients with diagnosed schizophrenia (130–132). Considering that binding of NCS-1 potentiates D2 receptor signaling, it is enticing to speculate that the increased NCS-1 levels result in supersensitivity to this receptor in schizophrenic patients, but experimental proof of this has yet to be provided. Ambiguous results regarding spinophilin levels show both increased and unaltered levels (131, 132). Several studies have reported elevated serum levels of the D2 receptor–interacting protein S100B to be associated with schizophrenia, and more specifically with negative symptomatology (133) and insulin resistance (134). Furthermore, treatment with antipsychotics suppresses both S100B and its scavenger, soluble receptor for advanced glycation end products (sRAGE) (135). Albeit these studies suggest a role for S100B in the etiology of schizophrenia, the exact nature and causal relationship have not been established.

Interestingly, several antipsychotics including haloperidol accumulate in lipid-raft fractions (136). Moreover, treatment with antipsychotics has been shown to affect cholesterol biosynthesis, lipid-raft composition, distribution of G proteins, and GPCR signaling. Haloperidol disrupts lipid rafts via inhibition of cholesterol biosynthesis, leading to increased accumulation of sterol intermediates, displacement of flotilin-1 and Fyn from lipid rafts, impairments in insulin-Akt signaling, and alterations in somatostatin receptor signaling and adenylate cyclase coupling (137, 138).

Atypical antipsychotics exert their effects by inverse agonism or antagonism of the 5-HT2A receptor in addition to antagonism of the D2 receptor. The adaptor protein PSD-95 has been shown to be crucial to the efficacy of such compounds (29). Phencyclidine (PCP)-induced disruption of prepulse inhibition (PPI) is a commonly used animal model for the positive symptoms of schizophrenia. Mice lacking the PSD-95 gene displayed a similar disruption of PPI in response to PCP as compared with wild-type mice, but the rescue of this phenotype that can be observed in wild-type mice following treatment with the atypical antipsychotic clozapine or two different

5-HT2A antagonists is absent in PSD-95 knockout mice. Similar results were obtained using PCP-induced hyperlocomotion, another animal model of schizophrenia.

The dopamine hypothesis of schizophrenia has long been a cornerstone in schizophrenia research, and even though additional neurotransmitters—notably glutamate—have been proposed to exert important functions (139–141), the D2 receptor remains an important target for pharmacotherapy of the disorder. Recent findings suggest that the antipsychotic effects of these compounds may involve disruption of the D2 receptor/β-arrestin signaling complex. Whereas different groups of antipsychotics have diverse effects on G protein–dependent D2 receptor signaling, they all seem to inhibit agonist-induced recruitment of β-arrestin 2 (110). Ligands that selectively inhibit G protein–independent signaling via the D2 receptor may provide efficient and specific antipsychotics.

Unipolar and Bipolar Depression

In addition to monoamines, depression involves alterations in stress responsivity, trophic support, amino acids, and neuropeptides. However, most of the current treatments against unipolar depression target monoamine transmission.

The fundamental role of 5-HT receptor signaling in depressive states is firmly established, but only a few studies have addressed adaptor proteins with regard to this topic. One notable example, however, is P11. This protein regulates 5-HT1B, 5-HT1D, and 5-HT4 receptor signaling, as described earlier in this review (21–23). Decreased levels of P11 mRNA were found in the cingulate cortex in a mouse model of depression, and they were also found in the same region in postmortem tissue from deceased patients diagnosed with unipolar depression. Per contra, treatment with tricyclic antidepressants or electroconvulsive therapy robustly increased P11 expression levels. In line with this finding, P11 knockout mice displayed increased depressive-like behaviors in several paradigms as well as reduced sensitivity to the antidepressant effect of 5-HT1B and 5-HT4 ligands and antidepressants (22, 23, 142). A recent study also proposed a role for P11 in the neurogenic effects of chronic antidepressants (142). This study reported that fluoxetine-induced neurogenesis was significantly reduced in mice lacking P11. Further analysis revealed that P11 is expressed not in neurogenic cells, but instead in adjacent GABA-ergic interneurons, which suggests that P11 exerts a neurogenic function by modulating 5-HT receptors on these cells. Together, these studies support a role for P11 in depressive states and also indicate that pharmacotherapies that induce P11expression or 5-HT receptor agonists that specifically favor recruitment of P11 to the receptor may provide efficient antidepressants.

In addition to P11, β-arrestin 2 may be involved in the antidepressant effects of fluoxetine (143). β-arrestin 2 was upregulated in the hypothalamus after chronic treatment with fluoxetine. Furthermore, the antidepressant response to such treatment was significantly reduced in mice lacking the β-arrestin 2 gene in several experimental measures of depressive-like behavior. β-arrestin 2 may also mediate part of the mood-stabilizing effect of lithium. Perhaps the most established mechanism of action for lithium in this respect is inhibition of GSK3β signaling (144). Beaulieu and coworkers (145) have suggested that in addition to this way of action, lithium promotes the disruption of a signaling complex consisting of β-arrestin 2, AKT, and PP2A and, in this way, inhibits downstream GSK3β signaling. The relevance of this interaction was further supported by in vivo studies showing that both molecular and behavioral manifestations of lithium treatment are altered in β-arrestin 2 knockout mice.

Many investigators have proposed a link between a hypodopaminergic state and depressive states. In agreement with this idea, some atypical antidepressants exert their effects via stimulation of the mesolimbic dopamine system, either directly via D2 agonism or indirectly via antagonism of

5-HT2C receptors (146). The adaptor protein prostate apoptosis response 4 (Par4) modulates D2 receptor signaling and has been implicated in depressive-like behavior (147). This adaptor protein binds to a calmodulin-binding motif in the third intracellular loop of the receptor and facilitates the inhibitory effect on cAMP signaling of D2 receptors. Calmodulin can compete with Par4 for this binding site and consequently can downregulate D2 receptor signaling. The interaction with Par4 is important for in vivo regulation of dopaminergic signaling because transgenic mice carrying a mutated form of Par4, where the D2 receptor interaction domain has been ablated, exhibit enhanced signaling via the dopamine-cAMP/CREB (cAMP response element binding protein) pathway as well as increased depressive-like behavior. This study (147) provides further evidence for impaired dopaminergic signaling in depressive-like states and suggests that adaptor proteins may play a crucial role in conveying such signaling.

The 5-HT transporter (5-HTT) is crucial for removal of 5-HT from the synaptic cleft and is a target for many antidepressants (148). Studies have shown that the 5-HTT localizes to lipid rafts both in vitro and in vivo and that association to these microdomains is crucial for 5-HTT activity, indicating lipid-raft localization as a novel regulatory mechanism for 5-HT uptake (149). Accordingly, several antidepressants are enriched in lipid-raft fractions (136). Further support for a role of lipid rafts comes from studies on postmortem tissue from suicide victims with confirmed unipolar depression; the studies reveal increased accumulation of $G_{s\alpha}$ in lipid rafts (150). These changes were observed in cerebellum and cortex and may affect the ability of $G_{s\alpha}$ to activate adenylate cyclase. In line with these data, several antidepressants including desipramine, escitalopram, and fluoxetine facilitate translocation of $G_{s\alpha}$ from lipid rafts and potentiate adenylate cyclase activity (151, 152). The latter studies are also in agreement with observations that rolipram, a cAMP phosphodiesterase inhibitor, has an antidepressant effect (153).

Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder that is characterized by degeneration of dopamine-producing neurons in the substantia nigra pars compacta. The behavioral features of Parkinson's disease include hypokinesia, rigidity, resting tremor, and gait abnormalities. In addition, Parkinson's disease is often associated with emotional and cognitive disturbances; of these, depression is the most frequent. Parkinsonism is treated by agents that, via different mechanisms, enhance dopamine neurotransmission. The gold standard is administration of a brain penetrant precursor to dopamine, L-DOPA, which is converted to dopamine upon entry in the CNS. However, L-DOPA treatment causes side effects, and dyskinesia ensues after prolonged treatment.

Following L-DOPA treatment, P11 expression and functional 5-HT1B receptors are increased in striatonigral neurons in the 6-hydroxydopamine model of Parkinsonism (154). The observed changes also appear to have functional consequences for the behavioral manifestations of L-DOPA treatment because treatment with a selective 5-HT1B agonist decreased L-DOPA-induced rotations and involuntary movements in a P11-dependent manner. These results point to an important role of 5-HT1B and P11 in regulating responses to L-DOPA and, together with the results from the studies on depression, emphasize the inducible and plastic regulation of P11.

CONCLUSIONS

A significant number of adaptor proteins that interact with monoamine receptors have been identified. Such interactions determine receptor surface expression, maturation from ER, sorting in

endocytotic vesicles, dendritic targeting, and G protein–dependent signaling. In addition, it has recently become evident that adaptor proteins play a crucial role in G protein–independent signaling. Adaptor protein interactions also play a role in psychiatric and neurological disorders, and two strategies that target such interactions have been devised: peptides that interfere with protein-protein binding, and biased ligands that affect recruitment of different adaptor proteins. Both these approaches are promising and have increased our knowledge of how adaptor proteins modulate GPCR signaling, but their helpfulness for developing novel pharmacotherapies for use in a clinical setting has yet to be tested. Several disease states with neurological manifestations show dysfunctions in the constituents of lipid rafts. Many monoamine receptors have a cholesterol consensus motif, which confers a direct interaction with cholesterol. There are also several lines of evidence, mainly from non-neuronal cells, suggesting that caveolins and lipid rafts significantly modulate monoamine receptor function.

SUMMARY POINTS

1. Studies have identified a vast number of adaptor proteins that interact with monoaminergic receptors. These proteins have been shown to influence surface receptor expression, G protein–dependent signaling, maturation from the ER, sorting of endocytosed receptors, and, in neurons, targeting of receptors to dendritic compartments.
2. Adaptor proteins such as GIPC and spinophilin regulate G protein–dependent signaling of adrenergic receptors by recruiting RGS proteins close to the receptors. The RGS proteins subsequently facilitate hydrolysis of bound GTP to GDP, thereby terminating signaling.
3. Monoamine GPCRs including the β_2-AR, D2, 5-HT2C, and 5-HT4 receptors can signal in a G protein–independent manner. Adaptor proteins play a key role in conveying such signaling, as exemplified by the β-arrestins. Characterization of additional adaptor proteins that facilitate G protein–independent signaling for monoamine receptors is certain to follow.
4. Two strategies for modulating receptor/adaptor protein interaction have emerged. The first involves interfering peptides that can directly interfere with and disrupt the interaction between the two molecules. The second approach involves biased ligands that can elicit specific responses in terms of adaptor protein recruitment, receptor trafficking, G protein–dependent signaling, and G protein–independent signaling.
5. Several adaptor proteins that interact with monoamine receptors have been implicated in disease states, including depression and mood disorders, schizophrenia, addiction, and Parkinson's disease. Some of these proteins are also important for the therapeutic actions of drugs used to treat these disorders.
6. Two recent lines of research have pointed to the importance of lipids in regulating monoamine receptor signaling and trafficking. First, several monoamine receptors have a cholesterol consensus motif that confers a direct interaction with cholesterol. Second, in many instances, caveolins and lipid rafts modulate monoamine receptor localization, internalization, and signaling.

FUTURE ISSUES

1. The application of new approaches (e.g., pull-down experiments coupled to mass spectrometry) will allow high-throughput identification not only of individual adaptor proteins but also of protein complexes that contain members not directly associated with the receptor. Challenges will lie in validating and attributing function to this wealth of interactions.
2. Development of perfectly biased ligands would greatly improve the knowledge of how G protein–dependent signaling and –independent signaling differ in how they mediate aspects of monoamine signaling. Another approach toward that end might involve correlating with physiological responses the agonistic or antagonistic properties on both pathways of currently used monoamine pharmacotherapies. Finally, identification of additional adaptor proteins that convey G protein–independent signaling is highly warranted.
3. The absolute majority of identified adaptor proteins that bind to monoamine receptors interact with receptors of the adrenergic, dopaminergic, and 5-HT systems. Much less is known about the other systems, including histamine, melatonin, and trace amine receptors, and further efforts in characterizing adaptor proteins modulating these receptor systems are required.
4. Our knowledge about the involvement of lipid rafts for monoamine receptor functionality in neuronal cells is still limited. In particular, the roles of cav-1 and cavins are poorly understood because most studies of their actions are performed in non-neuronal cells, which, unlike nerve cells, contain caveolae.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to apologize to all the authors whose work could not be properly cited because of space limitations. P.S. is supported by Vetenskapsrådet, the Royal Academy of Sciences, the Söderberg Foundation, and the Swedish Brain Fund.

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Volume 51, 2011

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