

G Protein-Coupled Receptor Signaling Complexity in Neuronal Tissue: Implications for Novel Therapeutics

Stuart Maudsley^{1,*}, Bronwen Martin¹ and Louis M. Luttrell²

¹Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Gerontology Research Center, 5600 Nathan Shock Drive, Johns Hopkins Medical Center, Baltimore, MD 21224, USA; ²Departments of Medicine and Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, USA; The Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29401, USA

Abstract: The manipulation of transmembrane signaling by G protein-coupled receptors (GPCRs) constitutes perhaps the single most important therapeutic target in medicine. Therapeutics acting on GPCRs have traditionally been classified as agonists, partial agonists, or antagonists based on a two state model of receptor function embodied in the ternary complex model. Over the past decade, however, many lines of investigation have shown that GPCR signaling exhibits greater diversity and 'texture' than previously appreciated. Signal diversity arises from numerous factors, among them the ability of receptors to adopt multiple 'active' states with different effector coupling profiles, the formation of receptor dimers that exhibit unique pharmacology, signaling, and trafficking, the dissociation of receptor 'activation' from desensitization and internalization, and the discovery that non-G protein effectors mediate some aspects of GPCR signaling. At the same time, clustering of GPCRs with their downstream effectors in membrane microdomains, and interactions between receptors and a plethora of multidomain scaffolding proteins and accessory/chaperone molecules confers signal preorganization, efficiency, and specificity. More importantly it is likely that alteration in the interactions of these proteins with GPCRs may occur in aging or neurodegenerative disorders, thus defining a distinct 'pharmacology' from that seen in young organisms or normal physiology. In this context, the concept of agonist selective trafficking of receptor signaling, which recognizes that a bound ligand may select between a menu of 'active' receptor conformations and induce only a subset of the possible response profile, presents the opportunity to develop drugs that change the quality as well as the quantity of efficacy and enhance these qualities for specific disorders or other paradigms. As a more comprehensive understanding of the complexity of GPCR signaling is developed, the rational design of ligands possessing increased specific efficacy and attenuated side effects may become the standard mode of drug development.

INTRODUCTION

The heptahelical G protein-coupled receptors (GPCRs) constitute the most diverse form of transmembrane signaling protein. Approximately 1% of the mammalian genome encodes GPCRs, and about 450 of the approximately 950 predicted human GPCRs are expected to be receptors for endogenous ligands [1-2]. GPCRs detect an extraordinarily diverse set of stimuli in the external environment, from photons of light and ions to small molecule neurotransmitters, peptides, glycoproteins, and phospholipids. Emphasizing their importance as therapeutic targets, nearly 40% of all current drugs target GPCRs for their actions [3].

The mechanism by which GPCRs transduce extracellular messages into intracellular cellular responses has long been envisioned as a simple linear model in which agonist binding promotes transition of the receptor from an 'off' to an 'on' state capable of engaging heterotrimeric guanine nucleotide-binding (G) proteins, whose dissociated G α and G $\beta\gamma$ subunits in turn activate or inhibit various downstream effector molecules. Implicit in this model is the concept that a

GPCR agonist will impact every consequence of receptor activation in the same fashion, whether G protein coupling, receptor desensitization, internalization or trafficking. Unfortunately this simple model has been overshadowed by experimental evidence documenting the existence of multiple 'active' receptor states, alternative mechanisms of signaling, and preorganization of GPCR signaling units. These findings have dramatically expanded our notions of the complexity and 'texture' of GPCR signaling and forced a re-examination of the fundamental concepts of agonism and antagonism. It is increasingly clear that for a given GPCR, the optimal receptor conformation for G protein activation differs between G protein pools, and that synthetic, and in some cases naturally-occurring, ligands can selectively promote different coupling conformations of the receptor (for examples see below). Many examples now exist of 'agonists' that activate only a subset of potential G protein partners or induce G protein coupling without triggering desensitization and endocytosis, or of 'antagonists' that cause receptor desensitization or that initiate apparently G protein-independent signals without producing detectable activation of heterotrimeric G proteins. Here, we examine current insights into the source of GPCR signaling diversity and specificity, and discuss the impact of these factors on the classical concepts of agonism and the process of drug discovery for neurological disorders.

*Address correspondence to this author at the Laboratory of Neurosciences & MedStar Research Institute, National Institute on Aging Intramural Research Program, Gerontology Research Center, 5600 Nathan Shock Drive, Johns Hopkins Medical Center, Baltimore, MD 21224, USA; Tel: 410 558 8472; Fax: 410 558 8323; E-mail: maudslst@grc.nia.nih.gov

THE EVOLUTION OF RECEPTOR THEORY

The Ternary Complex Model of GPCR Function

Ligand-mediated GPCR activation induces guanine nucleotide exchange at heterotrimeric G proteins. The ligand, upon binding, activates the receptor by causing conformational shifts within the heptahelical transmembrane domain bundle that are transmitted to the intracellular transmembrane loops and carboxyl terminus. These conformational changes alter the ability of the receptor to interact with intracellular G proteins and catalyze the exchange of GDP for GTP on the heterotrimeric G protein alpha subunit. The GTP-bound alpha subunit stimulates its cognate downstream effectors, *e.g.* an adenylyl cyclase or phospholipase C (PLC), conveying information about the presence of an extracellular stimulus to the intracellular environment.

When considered in the simplest way, a GPCR can act as a switch, existing in either an empty “off” state or an agonist-bound “on” state. Such early allosteric models of membrane receptor function were introduced in the late 1960s [4]. Even before the cloning of GPCRs it was shown that hormonal stimulation of adenylyl cyclase was specifically controlled by additional factors, *i.e.* guanine nucleotides [5]. The importance of these signaling factors in mediating receptor activation was reinforced with the finding that β -adrenergic receptors exhibit two affinity states for agonists and that the relative proportions of each state were modulated by the presence of guanine nucleotides [6]. This model advanced to explain these phenomena predicted that in the presence of GDP, agonist binding promotes the formation of a long-lived ternary complex between agonist (H), GPCR (R), and heterotrimeric G protein (G) that exhibits high agonist binding affinity. In the absence of the G protein, or when the presence of GTP allows for receptor-catalyzed G protein activation, the H-R-G complex is dissociated and the receptor resides in a low affinity (H-R) state.

The Extended Ternary Complex Model

The creation of chimeric receptors led to the demonstration that α 1b adrenergic receptors with relatively conservative substitutions of β 2 adrenergic receptor amino acid sequences in the C-terminal portion of the third intracellular loop could activate Gq/11 proteins in the absence of agonist [7]. In the limit case it was shown that mutation of a single residue, Ala293, of the α 1b-adrenergic receptor to any other amino acid increased the agonist affinity of the receptor and produced constitutive stimulation of phosphatidylinositol hydrolysis [8]. Using analogous constitutively active mutants of the β 2-adrenergic receptor, it was demonstrated that some ligands that appear to be classical competitive antagonists on native receptors show selective high affinity for the inactive receptor and suppress basal receptor activity [9]. Ligands possessing these properties have been termed “negative antagonists” or “inverse agonists”. This, and subsequent work involving a large number of GPCRs, led to the proposal that receptors exist in spontaneous equilibrium between two conformations (active: R*; inactive: R) that differ in their ability to activate G proteins [10]. In its native state the receptor occurs predominantly in the R conformation, maintained by intramolecular interactions within the transmembrane helical bundle, *i.e.* the spontaneous equilibrium heavily favors the

occurrence of the inactive R state. Agonist binding, relieves these constraints, allows the receptor to ‘relax’ into the R* conformation allowing G-protein coupling. The extended ternary complex model developed to explain these phenomena proposes that the intrinsic efficacy of a ligand (H) is a reflection of its ability to alter the equilibrium between R and R* [11]. According to this model, *Full agonists* stabilize the R* conformation, tilting the equilibrium to the active state, generating full receptor activation and a maximal response; *Partial agonists* have lower intrinsic efficacy than full agonists, producing a submaximal response and also potential attenuation of full agonist activation; *Antagonists* bind indiscriminately to both R and R*, producing no physiological response but blocking the response to agonists; *Inverse agonists* act as antagonists in non constitutively-active systems, but have the added property of actively reducing receptor-mediated constitutive activity of GPCR systems by binding preferentially to R and pushing the equilibrium toward the inactive state. Even the behavior of “Protean agonists”, ligands that act as partial agonists in some systems and as inverse agonists in others, can be accounted for within the extended ternary complex model if one assumes that the active receptor conformation produced by ligand binding (H-R*) is of a lower efficacy than that of the spontaneously formed R* state [12]. Under conditions of low basal activity, *i.e.* little or no spontaneously formed R*, such a ligand would behave as a partial agonist, while under conditions of high basal activity it would behave as an inverse agonist.

Three State to Multi-State Models

While the ternary complex model can sufficiently explain the properties of agonism, antagonism, partial agonism, and inverse agonism, it is still limited in that it accommodates the existence of only two functional receptor states. However several lines of experimental evidence suggest that multiple active states of GPCRs can exist. The first demonstrations of these were actually presented for the visual GPCR rhodopsin which was shown to adopt multiple spectral states [13, 14]. Many other GPCRs, either at physiologic levels or when overexpressed, are promiscuous in that they stimulate different signaling pathways by activating more than one G protein pool. In a two state model, where only a single R* conformation exists, the agonist pharmacology of a receptor should be the same regardless of the response being measured. Yet a paradoxical reversal of relative efficacy of agonists has been described for several GPCRs in the central nervous system that activate more than one stimulus-response element, including the 5-HT_{2C} receptor [15], pituitary adenylyl cyclase-activating polypeptide (PACAP) receptor [16] dopamine D₂ receptor [17], and neurokinin NK-1 receptor [18]. Striking discontinuities of agonist efficacy have also been reported for CB₁ cannabinoid receptor coupling to G_s and G_i [19]. Although differential stimulus pathway activation can occur through a ‘strength of signal’ type of mechanism, *i.e.* a highly efficacious agonist might activate two pathways whereas a weaker agonist may activate only the more sensitive one, the reversal of the relative efficacy of different agonists acting on the same receptor cannot be explained on the basis of a two state model.

In these three-state or multistate models, certain agonists are predicted to induce distinct “active” conformations of the

receptor by differentially exposing regions of the intracellular domains involved in coupling to different G protein pools [20, 21]. Indeed, multiple G protein-coupled states of the α_2 -adrenergic receptor can be distinguished using a variety of guanine nucleotide analogues [22]. Similarly, several receptor mutations have been described that produce constitutive activity that is restricted to a single signaling pathway among those ordinarily activated by the receptor [23, 24]. These mutations presumably restrict conformational isomerization of the receptor to a subset that promotes specific G protein coupling conformations [21, 25, 26, 27]. However these observations should be tempered with the caveat that distinct signaling varieties demonstrated using a mutated receptor may not be indicative of the true signaling potential of the receptor. Biophysical evidence also supports the concept that different GPCR ligands induce distinct populations of receptor microconformations [28, 29]. In these studies different agonists select distinct arrays of receptor conformation, consistent with the induction of ligand-selective active states.

The existence of multiple active receptor conformations makes it plausible that agonists can change not only the degree, but also the 'quality' of receptor activation. It is thus predictable that agonists producing distinct conformations of a receptor could expose different G-protein-activating sequences of the receptor so as to produce differential activation of G proteins. This multi-state model of GPCR activation provides the theoretical basis for the concept of signaling-selective agonism, also referred to as 'agonist-specific trafficking of receptor signaling' [12, 30].

THE ORIGINS OF GPCR SIGNALING DIVERSITY

In contrast to the large number of GPCR sequences in the genome, there are comparatively few genes encoding heterotrimeric G protein subunits [31]. Many elegant experiments however have demonstrated that signal diversity and specificity can be dictated by receptor interaction with specific heterotrimeric subunit combinations [32, 33]. This review shall focus upon the conditioning of GPCR signaling induced non-G protein mediated processes. With input from a large number of receptors converging on a limited number of transducer elements, how do GPCRs generate diverse responses in the nervous system under different conditions and in different tissues?

Diversity in a Two-State Model

Although classical receptor theory allows for only a single 'active' state of the receptor, numerous factors expand the signaling repertoire of heptahelical GPCRs. First is the sheer complexity of the receptors themselves. The majority of GPCR families consist of multiple receptor subtypes, often with different G protein coupling specificities. For example, there are at least 12 different mammalian genes encoding serotonin receptors. Additional complexity derives from alternative splicing of receptor genes and RNA editing, generating multiple receptor isoforms with distinct biochemical properties from the same gene [34].

Another layer of complexity arises from the ability of each G protein class to activate multiple downstream effectors. Both $G\alpha$ and $G\beta\gamma$ subunits contribute to the modulation, in a synergistic or antagonistic fashion, of either the

same or unrelated effectors, resulting in dual intracellular signaling. An example is the simultaneous G_i/o -mediated inhibition of adenylyl cyclase via the $G\alpha$ subunit and stimulation of PLC β via the $G\beta\gamma$ subunit [35, 36]. Further complexity arises from secondary modulation of intracellular effectors, for example the indirect activation of phospholipase A2 following a rise in intracellular Ca^{2+} concentration [37, 38].

Finally, there is the capacity for simultaneous activation of multiple G protein pools. Some G_i/o -coupled receptors, for example, mediate phosphoinositide hydrolysis through a pertussis toxin-insensitive pathway in addition to mediating pertussis toxin-sensitive inhibition of adenylyl cyclase [39, 40]. The dual coupling to G_s and $G_q/11$ family G proteins [41] or to G_i/o and $G_q/11$ family G proteins [42-44] has now been reported for many GPCRs. In some cases, a single receptor has been found to simultaneously activate members of three or even four unrelated classes of G protein (G_s , G_i/o , $G_q/11$, and G_{12}) [45].

A persistent question is whether multiple G protein coupling represents *pleiotropy*, i.e. physiologic activation of different G protein species, or *promiscuity*, i.e. low efficacy activation of non-preferred G protein species as a result of receptor or G protein overexpression. In experimental systems, an agonist activating one GPCR that stimulates multiple G proteins frequently elicits signals downstream of each G protein with differing efficacy and/or potency [43]. Unless there is reversal of agonist efficacy, such behavior is consistent with a two state model in which the receptor can interact with both preferred and secondary transducers. Indeed, emergence of a dual signaling commonly occurs as the level of receptor expression increases, e.g. pathologically, suggesting that most GPCRs are promiscuous [46, 47]. Similar phenomena arise from changes in the expression levels of the participating G proteins [48]. On the other hand, many studies have demonstrated dual or multiple coupling in systems where the GPCR is constitutively expressed at low levels, consistent with physiologically relevant pleiotropic G protein coupling [44, 49].

Diversity Due to Multiple Receptor Conformations

Consistent with models of agonist-specific trafficking of receptor signaling, a number of structurally modified agonists for promiscuous peptide and non-peptide GPCRs have been shown to promote selective G protein coupling [50, 51]. A similar phenomenon is signal-selective antagonism, in which an antagonist blocks only a subset of the signaling pathways elicited by an agonist. This has been clearly described for the cholecystokinin CCK-B [52] and neurokinin NK-1 receptors [53]. Other examples of ligand-selective GPCR regulation include ligands that promote coupling to one G protein pool while antagonizing coupling to another. The gonadotropin-releasing hormone (GnRH) receptor 'antagonist' Ant135-25 acts as an antagonist with respect to G_q -coupling by the GnRH receptor, but functions as an agonist in cellular contexts where the receptor is coupling to G_i [54]. Similarly, the β_2 -adrenergic receptor 'antagonist' ICI-118-551, which behaves as an inverse agonist for coupling to G_s [9], was recently found to act as an agonist for β_2 -adrenergic receptor coupling to G_i [55].

Studies of agonist-induced GPCR desensitization and endocytosis have likewise demonstrated the existence of ligand-specific receptor conformations. In a two-state model, it would be expected that the relative propensity of agonists to induce desensitization would parallel their relative efficacy for signaling. For μ opioid receptor agonists this is generally true, with the notable exceptions of methadone, L- α -acetyl methadone, and buprenorphine, which induce disproportionate receptor phosphorylation and desensitization [56]. Similarly, both enkephalins and morphine stimulate δ and μ opioid receptors, but only enkephalins induce rapid receptor internalization [57]. Disparities between primary pathway activation and desensitization have been also demonstrated for neurokinin NK-1 [58] and serotonin 5-HT_{2C} receptors [59]. Studies of the recovery from desensitization also suggest that agonists differentially affect receptor conformation. Whereas the resensitization of 5-HT₃ receptors after prolonged stimulation with partial agonists is mono-exponential, desensitization induced by full agonists recovers with sigmoid kinetics, suggesting at least 3 transitional steps and up to 4 states [60]. Even more dramatic are GPCR 'antagonists' that stimulate receptor internalization. The cholecystokinin (CCK) receptor antagonist D-Tyr-Gly-[(Nle28,31,D-Trp30)cholecystokinin-26–32]-phenylester, which blocks CCK-mediated G protein activation, nonetheless causes profound receptor internalization [61]. The finding that synthetic ligands can induce two or more functionally distinct receptor conformations suggests the possibility that native hormones interacting with the same GPCR may exhibit agonist-specific trafficking. Does this phenomenon occur in nature? Some data suggest so. For example CCL19 and CCL21, two endogenous chemokine Type 7 receptor ligands that exhibit equivalent potency and efficacy with respect to calcium mobilization differ dramatically in terms of their ability to cause receptor phosphorylation and desensitization [62].

It is therefore clear that GPCR ligands can condition the quality of the receptor activation event through control of structural reorganization. These ligands mentioned in this section typically interact with the classical ligand pore upon the receptor. This classical hormone-interacting site, typically in the helical bundle for small biogenic amines or on the superficial extracellular loops and regions of the helical core for small neuropeptides, is known as the orthosteric binding site. GPCRs, however, like ligand gated ion channels such as the GABA_A or NMDA receptor, possess additional ligand 'binding' sites that can allosterically affect receptor function. Compounds, different from the original cognate ligand, that interact with these allosteric sites on GPCRs can serve to potentiate or attenuate the activity of the endogenous ligand at the receptor [63]. Therefore the presence of such sites may additionally expand the diversity of signaling behavior that can be seen through GPCRs.

Diversity Arising from Receptor Dimerization

Co-precipitation studies, complementation experiments using mutated or chimeric receptors, and fluorescence energy transfer measurements all support the hypothesis that many, if not most, GPCRs can form homodimers, heterodimers, or higher order multimers [64]. The assembly of receptor multimers establishes another level of conditioning that

can affect GPCR ligand recognition, signaling, and intracellular trafficking. In the limit case, receptor dimerization is a prerequisite for the functionality of the receptor. The γ -amino butyric acid type B (GABA_B)R1 and GABA_BR2 receptors are nonfunctional as monomers. Only GABA_BR1-R2 heterodimers are capable of membrane expression and signaling [65]. Dimerization of the μ and δ opioid receptors decreases the affinity for certain agonists [66] whereas the converse is true for heterodimers of the adenosine A2A and dopamine D1 receptors, where selective agonist affinities are increased [67]. Agonist efficacy can also be altered by GPCR dimerization [68, 69]. For example, heterodimerization between somatostatin SSTR5 and SSTR1 and also between μ and δ opioid receptors, increases both the intrinsic efficacy and the apparent potency of some agonists [70].

Cross talk between heterodimeric GPCR pairs can positively or negatively modulate the response to agonist binding resulting in either enhanced G protein activation or cross-inhibition [71-73]. Even qualitative changes in G protein-coupling specificity have been reported. Whereas μ and δ opioid receptors couple to pertussis toxin-sensitive G-proteins when expressed individually, co-expression of these receptors results in opioid signaling in the presence of pertussis toxin [66]. Finally, heterodimerization can affect receptor desensitization and trafficking, thus modulating the duration of GPCR signaling, e.g. the nonselective opioid agonist etorphine, which causes internalization of δ , but not κ opioid receptors, does not cause δ opioid receptor internalization when it is co-expressed with the κ receptor [73].

Non-Receptor Modifiers of GPCR Signaling

The pharmacology of at least two neuronally expressed GPCRs is determined not exclusively by the structure of receptor alone, but by their interaction with the non-receptor RAMP (Receptor Activity Modifying Protein) and RCP (Receptor Component Protein) proteins [74, 75]. RAMPs form complexes with the calcitonin receptor-like receptor (CRLR) and calcitonin receptor and control receptor trafficking and function. The specific CRLR-RAMP complex determines the ligand specificity and expression of the receptor. The CRLR-RAMP1 complex acts as a receptor for the calcitonin gene-related peptides, a pleiotropic family of neuropeptides with homology to calcitonin, amylin and adrenomedullin. When CRLR is co-expressed with RAMP2 and RCP it functions as an adrenomedullin receptor. Similarly, complexes between a naturally occurring splice variant of the calcitonin receptor and RAMP1 or RAMP3 yields the functional amylin receptor. RAMP expression is modified under physiologic stress and in response to glucocorticoids, suggesting that cellular responsiveness to certain hormones can be regulated through the control of accessory protein expression [76-78].

Desensitization as a Modifier of Signal Quality

Mechanisms to dampen GPCR signals exist at multiple levels. At the receptor level, two processes, termed heterologous and homologous desensitization, respectively, have been shown to control not only signal duration and intensity, but also signal quality. In heterologous desensitization the activation of second messenger-dependent protein kinases,

such as protein kinase (PKA) and PKC, leads to phosphorylation of serine and/or threonine residues in the cytosolic loops and C-terminal tail of many GPCRs inhibiting receptor-G protein coupling. Agonist occupancy of the receptor is not required [79]. In contrast, homologous desensitization is specific for agonist-occupied GPCRs. It is a two-step process in which the receptor is first phosphorylated by one of a family of G protein-coupled receptor kinases (GRKs), then binds to an arrestin protein that exhibits high affinity only for the agonist-occupied, GRK-phosphorylated form of the receptor. Arrestin binding serves to both sterically inhibit G protein coupling and to target the receptor to clathrin-coated pits for internalization [80].

Receptor phosphorylation can alter the specificity of G protein coupling to a receptor *e.g.* PKA phosphorylation of S357 of the Gs-coupled prostacyclin receptor is required for alternative coupling to Gi and Gq/11 [81]. Other GPCRs demonstrate type selective desensitization of G protein coupling following PKA or PKC activation, *e.g.* Gq/11-mediated glutamate release by the subtype 1a metabotropic glutamate receptor (mGluR1a) is progressively desensitized by PKC-mediated receptor phosphorylation, while a simultaneous inhibitory signal mediated through Gi/o coupling remains unaffected. The result is that in the presence of a persistent stimulus, the mGluR1a receptor switches from an activator to an inhibitor of glutamate release [82]. Collectively, these data suggest that regulation of the G protein coupling specificity by receptor phosphorylation adds an additional level of control that permits the temporal resolution of cellular signaling elicited during the sustained stimulation of a receptor.

GPCR Coupling to Non-G protein Effectors

A final source of GPCR signaling diversity arises from data suggesting that GPCRs transmit 'G protein-independent' signals, and that coupling to certain non-G protein effectors exhibits features consistent with agonist-specific trafficking.

The intracellular domains of several GPCRs have been shown to bind to proteins that might function as alternative GPCR signal transducers, among them guanine nucleotide exchange factors (GEFs) for small G proteins, nonreceptor tyrosine kinases, and several proteins that function as adaptors or scaffolds [83]. A specific peptide motif in the C-terminus of the β 1-adrenergic receptor binds directly to the PDZ domain (named from Post synaptic density protein of 95 kDa (PSD95) - Discs large - Zona occludens proteins) domain of the cAMP-regulated Ras GEF (CN-Ras GEF), allowing the receptor to stimulate guanine nucleotide exchange on the small G protein, Ras [84]. Stimulation of the JAK-STAT pathway of transcriptional regulation by angiotensin AT1a receptors involves tyrosine phosphorylation of AT1a receptor tail by a Src family kinase, followed by association of JAK2 with the receptor. In this case, the binding of JAK2, which does not have a phosphotyrosine-binding SH2 domain, appears to be indirect, and may be mediated by a member of the SHP family of SH2 domain-containing tyrosine phosphatases [85].

However the most compelling evidence to date for 'G protein-independent' signaling involves the utilization of

arrestins as alternative signal transducers. The two non-visual arrestin isoforms (β -arrestin 1 and 2) can bind to several signaling proteins and recruit them to agonist-occupied GPCRs [86, 87]. Src family nonreceptor tyrosine kinases [88, 89], components of the c-Jun N-terminal kinase 3 (JNK3) and ERK1/2 MAP kinase cascades [90-92] and the PDE4D3 and PDE4D5 isoforms of cAMP phosphodiesterase [93] are recruited to GPCRs in this manner. In this distinctive model of GPCR signaling, β -arrestin binding is thought to confer enzymatic activity upon the receptor at the same time that it uncouples the receptor from its cognate G proteins. Indeed, the finding that arrestin-bound m2 muscarinic acetylcholine receptors (m2AChR) exhibit increased affinity for agonists, but not antagonists, has led to speculation that the agonist-receptor-arrestin complex represents an 'alternative ternary complex' [94]. For GPCRs, like the NK-1, AT1a, and V2 vasopressin receptors, all of which form stable GPCR-arrestin complexes, the β -arrestin-dependent 'signalsome' appears to remain intact as the receptor transits the endosomal compartment, resulting in activation of a spatially-constrained, extranuclear pool of activated ERK1/2 [92, 95]. In contrast, G protein-dependent ERK1/2 activation tends to promote nuclear translocation of the kinase and ERK1/2-dependent transcriptional responses. Thus signal strength, duration, subcellular localization, and functional consequence are all dictated by the mechanism of signal propagation.

THE ORIGINS OF SIGNALING SPECIFICITY

The converse of signaling diversity is signaling specificity, the constraint of responses to specific cells or nervous tracts, even when the stimulus itself may be neuromodulatory, as in the case of a secreted neuropeptide hormone. In essence, there must be mechanisms to limit 'signal spread' and work against promiscuity.

There are numerous factors, both intracellular and extracellular, with the classical conceptualization of GPCR signaling that promote specificity. For example, the highly localized release and rapid reuptake or extracellular degradation of neurotransmitters within the synaptic space provides a highly effective means of confining signals spatially. For neuromodulatory hormones, neuron-selective expression of receptor subtypes comes into play. A typical cortical neuron may express more than ten different GPCR genes, different combinations of G protein subunits, and multiple isoforms of effector molecules. The differential expression of these various proteins imposes signal specificity at many levels, resulting in hormone responses that are customized for the specific cell type. Signal duration and intensity are selectively modulated through rapid receptor desensitization and internalization, and more slowly by downregulation of receptor expression [96]. Nonetheless, GPCR signaling systems appear to exhibit higher levels of reorganization than can be accounted for by control of ligand availability and neuron-specific expression of transducer elements. Numerous studies have indicated that different GPCRs coupling to the same G protein in a single cell can elicit different biochemical or cellular responses [97-102]. A one-dimensional view of GPCR signal organization cannot readily account for such observations. At least two additional factors, compartmentalization of signaling proteins within membrane microdo-

mains and preorganization of GPCR signaling units through interactions with anchoring and scaffolding proteins, appear to play important roles in neuronal GPCR signaling specificity.

Specificity Arising within Membrane Microdomains

For most of the early years, GPCR signal transduction was conceptualized along the lines of a 'Brownian motion' model in which the random thermodynamic collision of signaling proteins within the plane of the plasma membrane was responsible for the flow of information from receptor to G protein to effector. Such a random process, however, would be energetically expensive for complex organisms that require rapidity and specificity of neuronal signaling function. Furthermore, mounting experimental evidence indicates that GPCRs, G proteins, and effectors are not randomly distributed in the plasma membrane. Indeed, it has been suggested that GPCR signaling mainly occurs within specialized microdomains, implying that the efficiency and specificity of signal transduction are dictated by the stoichiometry of transducer elements within spatially discrete membrane regions [103, 104].

One of the most studied forms of membrane microdomain in neuronal tissue are regions of high density cholesterol, gangliosides and sphingolipids referred to as caveolae or lipid rafts [105]. Many GPCRs and their associated signaling proteins, such as G proteins, RGS proteins and non-receptor tyrosine kinases like c-Src, have been shown to localize to these structures, often aided by C-terminal palmitoylation. A striking example of how localization of a GPCR within lipid microdomains can dictate signal selectivity is the neuropeptide oxytocin receptor. When present in caveolae the receptor exerts a proliferative effect upon HEK293 cells through a Gq-mediated mechanism involving cross talk with epidermal growth factor (EGF) receptors that also concentrate in caveoli. Oxytocin-stimulated EGF receptor 'transactivation' is independent of PLC, c-Src or phosphoinositide-3 kinase (PI3-K) activity. In contrast, activation of oxytocin receptors outside of rafts produces the exact opposite effect, an inhibition of cell proliferation that is Gi-, PLC-, c-Src- and PI3-K-dependent [106]. In addition to lipid rafts, other regions of the plasma membrane where signaling proteins are aggregated, such as focal adhesion complexes and clathrin-coated pits, appear to serve as sites of GPCR signal integration and specificity.

Scaffolding and Preorganization as Determinants of Signal Specificity

It is now clear that the intracellular domains of GPCRs participate in numerous interactions with cellular proteins that serve to organize the partners in a signaling cascade [107, 108]. In essence, these scaffolds assemble GPCRs, G proteins, effectors and downstream elements into prearranged 'solid-state' signaling devices that impose crucial spatial resolution and signaling compartmentalization on GPCR-mediated signaling systems. It is clear how important such organization is in complex cells such as cortical neurons, as their multiple regions have specific functions such as the soma, axon and terminal dendrites. For example, β 2-adrenergic receptors have a well documented association

with plasma membrane AKAP (A kinase anchoring proteins) proteins [109]. AKAPs act as dynamic platforms that orchestrate the interactions of protein kinases, including tyrosine kinases, protein phosphatases, *e.g.* calcineurin, and cytoskeletal elements with β 2 receptors. Other preformed complexes between the β 2 receptor and potential effectors have been reported, including association with the EGF receptor, a target for GPCR-stimulated 'transactivation' [110], and recently with the BKCa large conductance Ca^{2+} -dependent potassium channel [111].

Recently, a number of neuronal PDZ domain-containing proteins have been shown to interact with the distal C-terminus of select GPCRs and direct the assembly of functional synaptic protein networks [112]. This pre-organization of GPCRs into 'signalsomes' appears to be particularly prevalent within the central nervous system, where signaling efficiency and spatial constraint are paramount. Synaptically enriched proteins such as PSD-95, membrane-associated guanylate kinase inverted-2 (MAGI-2), SH3 multiple ankyrin domain-containing protein (Shank)/somatostatin receptor interacting protein (SSTRIP), Protein interacting with C kinase 1 (PICK-1), multi-PDZ domain protein 1 (MUPP1), and spinophilin contain between one and thirteen PDZ domains and all can associate with GPCRs forming higher order structures [113, 114]. Their association with many GPCRs, *e.g.* mGluRs, serotonin and adrenoceptors, has been reported to modulate such diverse functional properties as receptor dimerization, subcellular localization, effector coupling, and trafficking. For example the Homer proteins, which are involved in the control of actin filament dynamics, interact with polyproline sequences found in mGluR1 α , mGluR5 metabotropic glutamate receptor [115], Shank/SSTRIP, IP3 receptors [116], ryanodine receptors [117], and P/Q type calcium channels. Homer proteins function in the organization of postsynaptic glutaminergic sites, and excitation-dependent expression of Homer isoforms affects mGluR trafficking and targeting to axons and dendrites [118]. The dopamine receptor-interacting protein of 78 kDa (DRIP-78) binds to a C-terminal hydrophobic motif in D1 dopamine receptors and controls post-translational processing of the receptor [119]. The actin-binding protein 280 (ABP-280 or filamin A) interacts with the third intracellular (IC3) loop of the D2 and 3 dopamine receptors. ABP-280 binding fosters D2 receptor clustering at the plasma membrane and enhances the ability of D2 receptors to inhibit adenylyl cyclase. The 14-3-3 proteins, a family of at least seven acidic brain proteins that bind to phosphorylated serine/threonine motifs, interact with the third intracellular loops of α 2 adrenergic [120] and GABA_BR1 receptors [121] and appear to regulate GPCR dimerization, activation of the Ras/Raf cascade, and the localization of regulator of G protein signaling (RGS) proteins. Collectively, these examples illustrate the extent to which scaffolding protein interactions preorganize GPCR signals and ensure signal fidelity in neuronal systems.

GPCR SYSTEMS IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease known in industrialized nations, at least four million US citizens are currently afflicted with the disease. It is estimated that without effective prophylactic

treatment this number could rise to fourteen million by 2050 [122]. One major risk factor for AD is aging itself, however this is a natural process that is largely resistant to therapeutic intervention. The aging process involves complex changes in physiology resulting in great variations of expression or post-translational modification of a multiplicity of proteins. As we have seen in this review one must accept that GPCR function is dependent on many other protein factors outside the heptahelical core. Therefore the number of potential changes in GPCR functionality expands dramatically once one appreciates the myriad of protein factors that control GPCR pharmacology through the creation of discrete 'signalsomes' in the nervous system. While the processes that underlie the generation of hallmark AD pathologies, hyperphosphorylated tau and amyloid plaques, have been intensively studied, several important GPCR-related factors appear to play important roles in AD genesis and progression. With respect to the design of prophylactics or therapeutics, it is important to understand the potential changes in receptor pharmacology that may occur with aging itself and the pathological neurophysiological changes that occur in AD. An important caveat however needs to be inserted here in that we have shown earlier in this review that accessory proteins definitely can define GPCR signaling. However whether these alterations and their resultant effects signaling play an important and pervasive role in pathophysiology remains to be fully ascertained. We shall briefly discuss the relevance of GPCR factors in AD and then, with an eye to understanding how GPCR function is dependent on 'signal-some' complexity, attempt to rationalize novel therapeutic strategies to encompass this new model of pharmacology. Theoretically it should be possible to generate therapeutics with either an enhanced efficacy in certain physiological conditions, *e.g.* the aging patient, or selective efficacy for diseased states, *e.g.* AD, compared to normal physiological states.

A panoply of GPCR systems have been implicated in the etiology and pathology of AD. A small selection is shown in Table 1, however a caveat must be added with respect to lists larger than this, as these alterations in GPCRs could be merely reactive to the disorder and may not play an active role in its genesis. In the central nervous system (CNS), receptor signaling specificity and diversity has facilitated the creation of an unimaginably complex neurotransmitting system. In rudimentary terms the neurotransmission in the CNS consists of a small number of basic signaling modalities that are conditioned by a much greater number of subtle neuromodulating neurotransmitters. The primary signaling events controlling neuronal excitability occur through amino acid modulation of ligand-gated ion channels, *e.g.* excitatory glutamate (NMDA, AMPA and Kainate) receptors and inhibitory GABA_A receptors. Hormones acting at a multitude of GPCRs tend to exert more subtle neuromodulatory actions, adding 'texture' to the primarily 'binary' amino acid ligand-gated ion channel mechanism. The colossal variety, up to 60, of neuromodulatory agents, compared to the primary excitatory and inhibitory amino acids, hints at their importance in generating signaling complexity in the CNS. It is clear that many of the GPCR systems that possess a role in AD can show specificity of expression, *e.g.* dendritic, post- or pre-synaptic etc. The selectivity of subcellular expression likely

facilitates the generation of multiple GPCR receptorsomes that confer disparate functions to the same heptahelical core. Clearly, as seen in (Table 1), a vast array of GPCR-based systems could be involved in the aetiology of AD, yet for the purposes of this review we shall focus on the latest data on the more pharmacologically tractable targets with respect to our new knowledge of how receptor specificity and signaling diversity is generated.

Unfortunately for traditional receptor biochemistry two of the major classical targets of pharmacological intervention for AD, the muscarinic acetyl choline (ACh) and glutaminergic receptor systems consist of multiple receptor subtypes that bind the same small endogenous ligands, ACh and glutamate, with great similarity. In addition to these traditional therapeutic targets several new lines of evidence have shown the importance of the serotonergic GPCR system in AD. As with muscarinic and glutaminergic GPCRs there are a plethora of serotonin receptor subtypes present in the brain which all again bind serotonin with similar affinity. Therefore generation of signaling- and subtype-specific agents has proved to be difficult due to the commonality of ligand-receptor interactions at heptahelical cholinergic muscarinic, serotonergic and glutaminergic GPCRs. We shall discuss how understanding of how GPCR structure and function may be altered with accessory protein binding the creation of tissue-specific or even pathology-specific compounds may be facilitated.

CHOLINERGIC GPCR SYSTEMS IN ALZHEIMER'S DISEASE

One of the primary neurophysiological alterations that appears to occur in AD is the loss of cholinergic function in cortical and hippocampal brain areas [123]. Therefore the muscarinic receptors (m1-m5AChR subtypes) have been implicated in controlling the regulation of memory, learning processes and cognition. The cholinergic hypothesis of Alzheimer's has been exploited with therapeutics targeted towards elevating the pathologically lower levels of ACh observed in AD patients [for review see 124].

For example the expression of pre-synaptic m2AChR, an autoreceptor that controls ACh release, appears to be altered in AD [125, 126]. Current treatments for AD often involve increasing acetylcholine levels in the synapse through administration of acetylcholinesterase inhibitors. An alternative approach would be the administration of selective m2AChR antagonists to elevate the levels of ACh in the synapse. If the pre-synaptic m2AChR was held in a different conformation than others expressed in different cellular locations it should be possible to generate pre-synaptic-specific therapeutics.

With respect to the m1AChR, selective activation could provide potential benefit through a variety of mechanisms, *e.g.* amelioration of the cholinergic neurotransmission deficit, activation of α -secretase (non-amyloidogenic APP processing) and inhibition of tau phosphorylation [for review see 127]. Muscarinic receptor signaling in general can also affect beta-amyloid cleaving enzyme (BACE1) function. Selective stimulation of m1/m3AChRs elevates expression of BACE1 while stimulation of m2AChRs reduces the expression of BACE1, thus confirming from another angle the potential value of m2AChR inhibition [128]. Ameliorating the cho-

Table 1. GPCR Alterations Observed in Alzheimer's Disease and Pathology

Receptor Type	Alteration	Location	Functional Impact	Reference
m4AChR	Downregulated	Hippocampus	Increase AD symptomology in post-menopausal women	[172, 173]
m2AChR	Upregulated	Frontal Cortex	Increased inhibition of ACh release	[125]
Angiotensin AT1 receptor	Upregulated	Cortex-layer V	Microvascular pathology and AD symptomology	[174]
Alpha2-adrenoceptors	Downregulated	Pre-frontal cortex	Positively correlated with AD	[175]
Dopamine D2R	Downregulated	Temporal lobe	Decreased memory performance	[176]
Opioid receptors	Downregulated	Hippocampus CA3	Positively correlated with AD	[177]
Somatostatin SSTR 2, 4, 5	Downregulated	Cortex	Positively correlated with AD	[178]
Somatostatin SSTR 3	Upregulated	Cortex	Positively correlated with AD	[178]
Serotonin 5HT2A	Receptor polymorphisms	Cortex	Linked to psychotic aspects of AD	[135]
Serotonin 5HT1B/1D	Downregulated	Frontal cortex	Increased AD pathology	[141]
Serotonin 5HT6	Downregulated	Frontal cortex	Alteration of GABAergic inhibitory signaling	[141]
GABA _B receptor	Downregulated	Hippocampal CA1 and dentate gyrus	Positively correlated with AD	[179]
Galanin GAL1R	Upregulated ?	Basal forebrain	Decreased ACh neurotransmission	[180, 181]
Neuropeptide (NPY) receptor	Reduced activity	Cortex	Linked to AD symptomology	[182]
Melatonin 2 (MT2) receptor	Downregulated	Hippocampus	Positively correlated with AD	[183]
Cannabinoid 1 (CB1) receptor	Attenuated G protein coupling	Cortex	Positively correlated with AD	[184]
Metabotropic glutamate receptors	Downregulated	Hippocampus	Positively correlated with AD	[145]
Neurotensin receptors	Downregulated	Hippocampus CA3	Positively correlated with AD	[178]
Histamine H1	Downregulated	Frontal, Temporal cortex	Positively correlated with AD	[185]
Endothelin ET-1 receptor	Downregulated	Cortex, hippocampal CA1	Positively correlated with AD	[186]
Tachykinin NK1 receptor	Downregulated	Cortex	Attenuation of stimulated ACh release: Positively correlated with AD	[140]
Chemokine receptors	Upregulated	Cortex	Neuroinflammation	[187]

linergic signaling deficit in AD m1AChR activation may also directly attenuate the deleterious effects of A β peptide upon neurons. m1AChR-mediated inhibition of GSK3 β can stabilize cytoplasmic β -catenin resulting in an elevation of the *Wnt* target genes, engrailed and cyclin D1, that functionally antagonize the inhibition of the *Wnt* pathway induced by A β peptide [129]. Selective m4AChR receptor stimulation may even have a beneficial action on the psychotic components of AD [130]. An understanding of how the similar mAChRs are organized into superstructures may facilitate the ability to design agents that act at specific subtypes in particular subcellular locations.

Reinforcing its importance in AD the cholinergic system is subject to a great degree of modulation by other GPCR systems. Endogenous modulation of the cholinergic transmitter system in the hippocampus, the primary region of AD pathology interest, is primarily mediated through GABAergic mechanisms. It has been previously demonstrated that the G protein-coupled GABA_B receptor may represent an important target for therapeutic design as early GABA_B receptor antagonists show a capacity to improve cognitive function in a variety of animal models [for review 131]. Recently a specific GABA_B receptor antagonist, SGS742, has shown therapeutic promise by improving cognitive function in a double-blind Phase II trial of patients with mild cognitive impairment [132]. As we have discussed it is likely that there are many functional GPCR homodimers or heterodimers that contribute to the 'true' receptor pharmacology phenomena that has been observed. As GABA_B receptors exist functionally as dimeric molecules they present an excellent target for the creation of novel ligands that specifically recognize this new state of the molecule and may possess a whole new pharmacology compared to existing GABAergic agents. The screening process for compounds with specific activities at dimeric receptors however may necessitate the creation of covalently linked receptors to negate the presence of non-dimeric receptor forms. It is possible therefore that the creation of new ligand binding epitopes, ortho or allosteric, may occur with dimerization that could allow a novel form of control of receptor activity.

The actual connectivity of mAChRs with their G protein and other signaling systems are significantly downregulated in the frontal cortex of AD patients suggesting an alteration in the physicochemical structure of the receptor signalsome itself, 'decompartmentalising' it from its transduction machinery [133]. In addition to alterations of m2AChR signalsome structure, with the m1AChRs there is a disruption of the compartmentalization of its connection to its cognate RGS protein and Gq/11 protein [134]. The AD pathogenic alteration of the amyloid processing pathway itself may serve to modify GPCR signaling systems, as it has been demonstrated that mutations in presenilin 1 (PS1) can functionally attenuate intracellular phospholipase C signaling induced by muscarinic receptor activation, potentially contributing to the effective cholinergic deficit associated with AD [135].

Not only is the GPCR connection to signaling machinery in its receptorsome impaired in neurodegenerative diseases but also the regulatory processes affecting GPCRs have been implicated. For example, plasma membrane GRK2/5 activity

is significantly reduced in pre-symptomatic AD mouse cortex [136]. This loss of regulation may result in cellular hyperstimulation by numerous GPCR inputs. One example of hyperstimulation is excessive proteinase activated receptor (PAR)-mediated microglia activation or tau hyperphosphorylation in hippocampal neurones [137]. Proteinase-activated receptors (PARs) potentially play a dual role in promoting both neuronal survival and causing neurodegeneration. Ligands activating the PAR receptors have been shown to protect astrocytes and neurons [138]. There has been a significant degree of interest in the β -arrestin-dependence of PAR receptor signaling since connections between the receptor and its signaling scaffolds determines the eventual nature of the transduced signal. Excessive stimulation may entrain enhanced β -arrestin inclusion into the receptorsome causing a dynamic disruption of the correct multi-protein complex stoichiometry and re-direction of signaling into G protein-independent pathways.

SEROTONERGIC GPCRS IN ALZHEIMER'S DISEASE

Recent evidence has suggested that compromised serotonergic function, in addition to its well-known role in depression, may play an important role in cognitive decline in aging, AD and also schizophrenia. Complex changes appear to occur to 5-HT receptor systems in AD, which could open up avenues for the development of therapeutic agents to control AD symptomology. Serotonergic receptors have been found to occur in large receptorsome structures and it is highly likely that alterations in the receptor structure could affect the stoichiometry of these entities. Of particular interest is the observation that polymorphisms of the 5HT_{2A} receptor are strongly correlated with the presence of psychotic symptoms in AD [139]. Subtle changes in the helical core structure introduced by these polymorphisms may not significantly affect ligand binding and G protein signaling but may facilitate the creation of polymorphism-specific 5HT receptorsome complexes. Hence the change in structure of the receptor may only affect accessory protein binding, creating a specific receptorsome complex that possesses distinct signal transduction patterns compared to wild-type receptor complexes.

Serotonergic signaling systems have been shown to productively interact with the cholinergic signaling system in the brain, thus reinforcing their importance for AD research. Activation of somato-dendritically located 5-HT_{2A} receptors facilitates substance P (SP) release. SP, in turn, stimulates hippocampal ACh release through activation of tachykinin NK₁ receptors present on cholinergic terminals [140]. Pertinent to development of novel serotonergic AD therapeutics, several receptor subtype-specific perturbations of serotonin receptor function have been observed in AD. For example 5-HT_{1B/1D} and 5-HT₆ receptors are downregulated in the frontal cortex of AD positive patients [141]. Importantly the 5-HT₆ receptor appears to be predominantly post-synaptic in its expression and may be concentrated in GABAergic spiny neurons, which exert potent inhibitory actions upon excitatory glutaminergic neuronal circuits. Recent evidence has also suggested that selective 5HT 1A, 4 and 7 ligands can enhance memory formation and thus may form the basis of

future cognitive therapeutics [142, 143]. For example, Lecozotan is able to mediate an enhancement of stimulated glutamate and ACh release through selective inhibition of 5HT_{1A} receptors, resulting in a positive cognitive-enhancing action [144].

Generation of discrete receptor- and signaling-specific agonists against various 5HT-receptor subtypes may enhance the efficacy of serotonergic compounds for AD. Due to the complexity of the 5HT deficits/dysregulation in AD rather than using a series of 'selective' compounds it may be prudent to develop individual 'pluri-protean' serotonergic agents that display a variety of 'pharmacologies' dependent upon the type of neuron expressing the 5HT receptor and also the specific 5HT receptor subtype. Multi-receptor modulatory agents, that have agonistic capacities on some receptor subtypes and antagonistic actions on others may represent a more efficacious mode of therapeutic development.

METABOTROPIC GLUTAMATE GPCRS IN ALZHEIMER'S DISEASE

Neuronal excitotoxicity has been proposed to be involved in the pathogenesis of chronic neurodegenerative disorders, such as Parkinson's and Huntington's diseases as well as AD. It is accepted that modulation of the stimulatory glutaminergic neurotransmission could be used as a direct therapeutic for these disorders. Hence metabotropic glutamate receptors (mGluRs) may be relevant targets for intervention of neurotransmitter-mediated excitotoxicity in degenerative disorders. There are up to eight different subtypes of metabotropic glutamate receptors typically expressed in the CNS. All of these members have a high degree of amino acid conservation in the ligand binding pocket and thus bind glutamate in a similar manner between the multiple subtypes. These secretin-like Class II GPCRs control neural excitability and also neurotransmitter secretion. A down-regulation of mGluR binding sites has been reported in post-mortem AD brains [145]. The possibility of a protective role for the mGluR system in the nervous system has led to significant interest in agents that control glutaminergic signaling. mGluR activation prevents and, in some cases, reverses genomic DNA degradation [146], regulates the metabolism of APP, accelerates the processing of APP into non-amyloidogenic APP [147] and can enhance the release of the neurotrophic secreted form of processed amyloid precursor protein (sAPP) [148]. Reinforcing the recurring theme of GPCR signaling complexity, under some circumstances, diminished activity of mGluRs may even prove useful for cellular protection. For example, inhibition of group II mGluRs can attenuate microglial activation and subsequent neurotoxicity during toxic stimuli such as chromogranin A [149], a protein up-regulated in Alzheimer's disease. mGluRs are also believed to be necessary for the processing of learning and memory [150]. Activation of pre-synaptic group II/III mGluRs can inhibit glutamate secretion, reducing the neuronal excitotoxicity linked to excessive glutamate release. Therefore, selective mGluR agonists may provide neuroprotection and neuronal restoration by decreasing excitotoxic events and enhanced neurotrophic support. On the other hand, selective mGluR antagonists might alleviate deficits in

synaptic transmission in AD by preventing this inhibitory feedback on glutamate release.

Interestingly, mGluRs are expressed and function specifically as dimeric molecules tethered together by their large extracellular N-termini. Full receptor activation requires two glutamate molecules interacting with both receptors of the dimer. Monomeric activation can occur but does not yield the full spectrum of receptor activation seen with dimeric activation [65]. Therefore hybrid molecules containing two different entities, either fully or partially agonistic or even antagonistic, could be created using small molecular linkers between the two modulating ligands.

Metabotropic glutamate receptors can not only homodimerize but can also form functional heterodimeric signaling units with other GPCRS, *e.g.* adrenoceptors, adenosine receptors (A_{2A}), dopamine receptors (D2) and muscarinic receptors [for review see 151]. The potential to create hybrid multi-functional ligands, that differentially activate one or both of the pharmacologically-distinct dimerized receptors, is yet another test-bed for novel drug discovery strategies.

Drug discovery over recent years has focused upon the development of selective ligands that manipulate the target receptor by interacting with the epitopes on the receptor that the endogenous ligand interacts with, *i.e.* the 'orthosteric' modulatory site of the receptor. As mentioned earlier, it has proven problematic to generate mGluR ligands that are selective and specific in action due to the high level of sequence conservation between the eight mGluR subtypes in the agonist binding pocket and glutamine binding affinities. This, along with the difficulty of delivering charged amino acids through the blood-brain barrier, has hampered the development of 'orthosteric' mGluR-interacting ligands and the potential therapeutic benefit of such compounds remains to be realized. In contrast small inorganic compounds that bind allosterically to the GPCR have been shown to control GPCR activity in both synergistic and antagonistic capacities. Higgins et al. [152] found that LY354740, a positive allosteric regulator of mGluRs, impaired learning in the Morris Water Maze model, whereas an antagonist (LY341495) improved acquisition of spatial learning. In line with this, group I mGluR antagonists/negative allosteric modulators may show promise for neuroprotection in AD.

The importance of creating allosterically-acting GPCR ligands may be critical for AD therapeutics as the primary targets, *e.g.* mAChR, mGluR and serotonin receptors, have demonstrated a resistance to generating subtype-selective orthosteric ligands. Allosteric modulators tend to only have effects in the presence of the orthosteric ligand, *e.g.* ACh, glutamate or 5-HT, however such agents may have a specific advantage for the treatments of disorders characterized by neurotransmission deficits such as AD. Such modulators might selectively enhance the effects of the endogenous GPCR ligand without disrupting the normal temporal and spatial profile of neurotransmitter release. Therefore subtle memory or motor patterns, based upon existing neurotransmission circuits would likely be retained. With respect to AD, the failure of orthosteric compounds as effective therapeutics may be due to a disruption of the release patterns of the endogenous agents that entrain proper neurophysiological activity. This 'artificial' modulation of neurotransmitters

is more likely to induce desensitization or downregulation of the receptor systems they are attempting to modulate as the compounds may be continuously present rather than occurring at the correct synaptic concentration for the correct period of time. Therefore it is likely that true sub-type selectivity of muscarinic, glutaminergic or serotonergic agents may only be generated by targeting them to regions of the receptor protein outside of the highly-conserved orthosteric binding site. This seems feasible as the majority of allosteric modulatory sites in GPCRs occur in the less well conserved regions of the receptor, *i.e.* the extracellular loops and the superficial regions of the transmembrane helices. Many allosteric modulators that selectively modify signaling by receptor subtypes expressed in the CNS, *e.g.* mGluR 1, 4, 5; mAChRs, 5HT type 1B, 1D, 2A, 2C and 7 receptors, have been developed [153]. It is hoped that these agents could form the building blocks for the next generation of AD therapeutics.

THERAPEUTIC IMPLICATIONS OF RECEPTOR THEORY

Data continues to emerge indicating that GPCR signal transduction is both more diverse and more specific than originally imagined. The existence of multiple 'active' receptor states, of receptor-receptor and receptor-scaffold interactions that modify receptor pharmacology, of possible 'G protein-independent' signaling, and of tissue selective preorganization of signals, presents the opportunity to develop drugs that induce only a subset of the GPCR response profile. At the same time, signaling complexity implies the existence of pitfalls arising from unintended drug action. Not only do these additional protein factors allow specificity of signaling in the CNS but they also may underpin the generation of physiological phenotypes such as the aging process or be part of the etiology or the reactive response to neurodegenerative processes. The influence of external factors on character of GPCR signaling may generate diverse receptor pharmacologies that can engender the derivation of entirely new therapeutic pharmacopeias specifically tailored to these individual states, thus creating the field of 'expetopharmacology' from the latin for 'to design for'.

The potential therapeutic implications of agonist-selective signal trafficking extend beyond just the regulation of the receptor-G protein coupling event. For example, ligands that selectively induce receptor activation without inducing any significant internalization could be beneficial in treatment of psychopharmacological drug tolerance [56, 154]. Similarly, selective GPCR internalization may prevent HIV-1 infection through chemokine receptor fusion. Ligands that cause internalization of CXCR4 [155, 156] or CCR5 [157, 158] have been shown to protect against HIV-1 infection *in vitro*. Selective removal of chemokine receptors from the cell surface could be superior to blocking chemokine receptor interaction with HIV viral coat proteins because it would prevent the possible rapid emergence of resistant HIV variants through therapeutic pressure and mutation [159-161]. Receptor dimerization may also generate therapeutic targets with unique pharmacology and signaling characteristics. Receptor dimers have been implicated in numerous areas including HIV-1 infection [162, 163] and the function of neuroprotective cannabinoid receptors [164], GABA-B re-

ceptors [165, 166], adenosine A1 receptors [167], δ -opioid receptors [168], β_2 -adrenoreceptors [68], and calcium-sensing receptors [169]. Drugs that selectively target unique ligand-binding pockets generated through dimerization may produce effects not associated with monomeric receptor signaling. Finally, the recently discovered ability of ligands to selectively activate non-G protein-mediated signaling through GPCRs may prove therapeutically relevant.

The concept of agonist selective trafficking of receptor signaling has received much attention as it prompts the search for drugs that can change the quality as well as the quantity of efficacy [26]. It is now clear that even the terms agonist and antagonist are strictly context-dependent. If a ligand can discriminate between multiple 'active' receptor conformations to preferentially activate a subset of effector pathways, then agonist efficacy needs to be defined in terms of the assay used to measure receptor activation. In the broadest sense, all ligands that productively engage a GPCR have the potential to be 'pluri-protean', acting as both agonist and antagonist depending on the signaling function measured and the nature of the cellular environment. In some cases it may be useful to re-classify compounds based on a full profile of stimulus-response coupling. Separating agonists in this manner could offer insights into preferred profiles of agonism as compounds progress from screening assays into therapeutically-oriented secondary assays. In situations where an original screening program was limited to measuring a single signaling pathway, consideration should be given to re-examining the properties of some compounds that were initially disregarded on the basis of apparently poor efficacy [170]. As ongoing work provides greater insights into the multitude of factors that give texture to GPCR signaling, the challenge will be to exploit the complex behavior of these receptors for therapeutic advantage while minimizing the pitfalls associated with too narrow a vision of receptor function.

CONCLUSIONS

For the majority of its experimental lifetime, information flow through GPCRs has been envisioned as unidirectional, *i.e.* changes in receptor conformation produced by agonist binding promote the transfer of information from outside the cell inwards. Recent experimentation, however, has demonstrated that receptor conformation is also controlled by protein-protein interactions occurring inside the cell. Receptor dimerization and interactions with scaffolding and signaling proteins can modify ligand selectivity and predetermine, from a menu of available options, which intracellular responses will predominate. In essence, the influences on receptor conformation are bi-directional; internal factors change the conformation of the receptor to reflect the status of the intracellular milieu, while extracellular factors, *i.e.* agonists, convey information to the cell about the external environment. This concept has critical implications for receptor theory and the design of therapeutics. Thus in complex physiological processes, *e.g.* aging or neurodegenerative disease, in which multiple protein expression patterns are changed it is more likely than previously thought that GPCR signal conditioning could be affected. Therefore if indeed there is an alteration of GPCR pharmacology in these states then perhaps drug design should be targeted toward

this new pharmacology rather than the standard models previously used.

If one accepts the premise that the association of GPCRs with intracellular proteins places a constraint on the array of 'active' conformations that the receptor can adopt, then even

within a single cell there may exist different 'flavors' of the same receptor, pre-wired to produce specific responses to preferred ligands. In the limit case of one neuron expressing multiple copies of the same receptor it seems unlikely that every copy of the receptor would be coupled to the same signal transduction machinery at all times. This is certainly

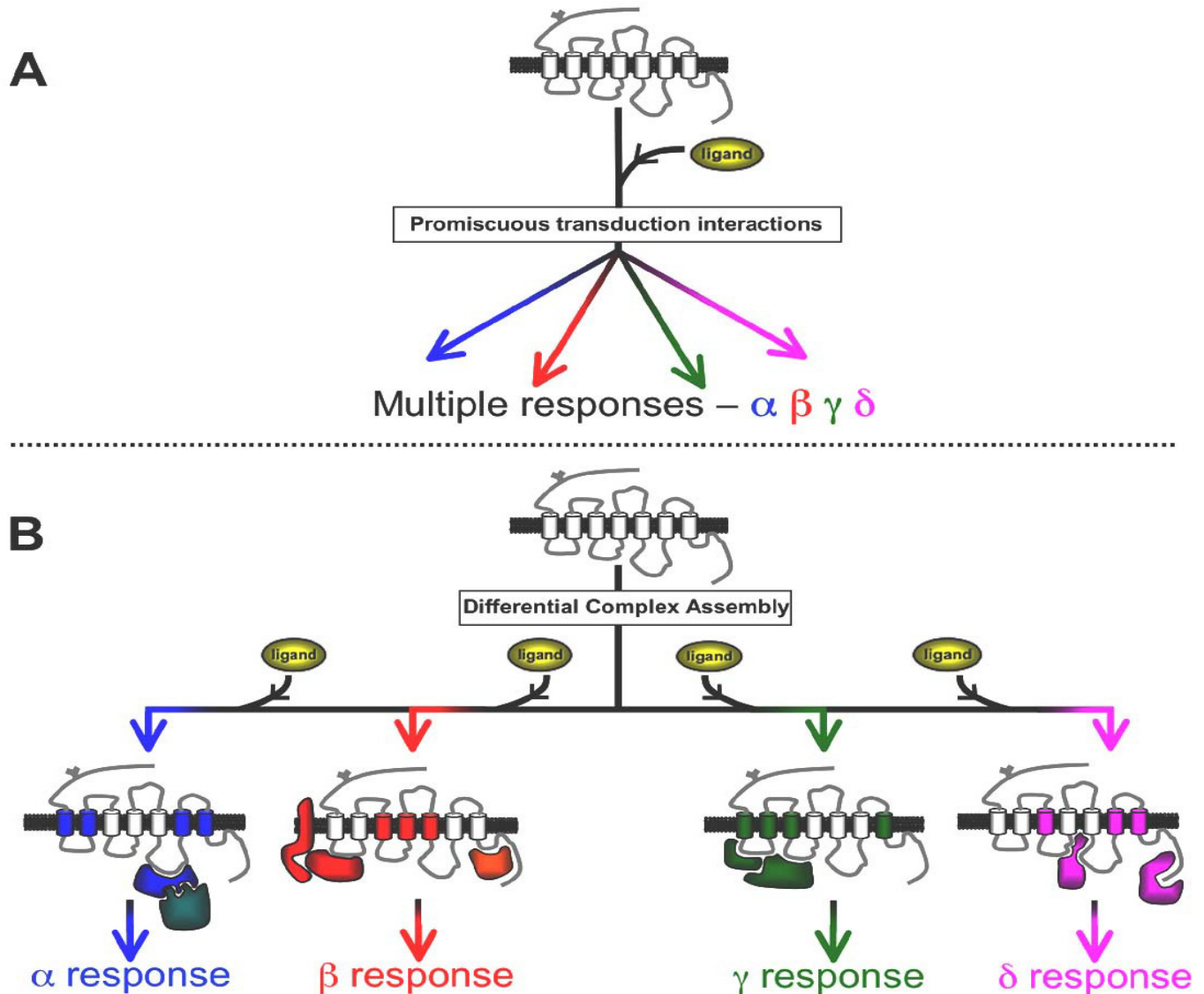


Fig. (1). Differential receptor complex pre-assembly allows efficient and rapid activation of multiple signal transduction pathways. In panel A we consider a single isoform of a heptahelical GPCR. Upon ligand stimulation the receptor can activate multiple intracellular signal transduction pathways resulting in different responses, α , β , γ and δ . In this scenario the ligand interacts with a base inactive state receptor and then mediates promiscuous coupling with various downstream signaling factors to induce the multiple responses. In contrast however, in panel B we consider that the stable association of the base, inactive state of the receptor with various accessory proteins results in the generation of various stable forms of the receptor, *i.e.* the differently colored 'flavors'. The tertiary structure, isomerizational capacity (between active and inactive states), connection to downstream transduction pathways and eventual responses of each of the GPCR 'flavors' is a function of the coterie of accessory proteins that constitute it. Therefore the ligand can bind to differing 'flavors' of the receptors that are pre-disposed to activate certain signaling outcomes. If indeed the endogenous ligand may have multiple structural forms, or the receptor binds multiple endogenous ligands, it is conceivable that a ligand selection event may take place at the different receptor 'flavors'. Due to the different tertiary structures and isomerizational capacity imparted on the helical core by the accessory proteins, ligand selectivity may be induced into this receptor system so that certain ligands only induce specific signaling events. Thus with ligand selection occurring due to the differential receptor complex formation there is the generation of distinct intracellular signaling responses, despite the central receptor helical cores being identical in their primary sequence.

true if the receptor is susceptible to G protein 'switching' induced by heterologous desensitization [171], or capable of signaling through β -arrestins [86, 87]. These various receptor 'flavors' could preferentially interact with different ligands, e.g. CRLR-RAMP1 recognizing calcitonin gene-related peptides and CRLR-RAMP2-RCP binding adrenomedullin [74, 75], or activate different downstream effectors in response to the same ligand. We have recently invoked the latter scenario with respect to tissue-specific differences in GnRH receptor signaling [54].

An interesting corollary of this postulate is that GPCRs may not be 'truly' promiscuous in the sense of a single receptor interacting with multiple effector pathways in a random manner. If GPCRs can be preassembled with their downstream transduction machinery, it may be biophysically more efficient to generate a variety of receptor 'flavors' that are hard-wired to specific transduction pathways than to switch a single receptor between different pathways (Fig. 1). Such a model could accommodate experimentally observed promiscuity if the primary response pathway is defined as that mediated by the receptor 'flavor' with the highest affinity for a given agonist, whereas promiscuous coupling results from the activation of alternative receptor 'flavors' that have lower affinity for the ligand and therefore are only activated by higher agonist concentrations. Reversal of agonist efficacy could similarly result from altered ligand selectivity imposed from inside the cell through protein-protein interactions affecting ligand affinity. While selective examples of each of these phenomena exist, additional experimentation will be required to determine whether these mechanisms have broad applicability to models of GPCR signaling.

ABBREVIATIONS

EGF	=	Epidermal growth factor
ERK1/2	=	Extracellular signal-regulated kinases 1 and 2
GPCR	=	G protein-coupled receptor
G protein	=	Heterotrimeric guanine nucleotide-binding protein
GEF	=	Guanine nucleotide exchange factor
MAP	=	Mitogen-activated protein
NHERF	=	Na^+/H^+ exchanger regulatory factor
PDZ	=	Post synaptic density of 95 kDa-disc large-zona occludens
PK	=	Protein kinase
PLC	=	Phospholipase C
RAMP	=	Receptor Activity Modifying Protein
RCP	=	Receptor Component Protein

REFERENCES

- [1] Hur EM and Kim KT. G protein-coupled receptor signaling and cross-talk: achieving rapidity and specificity. *Cell Signal* 14:397-405 (2002).
- [2] Takeda S, Kadowaki S, Haga T, Takaesu H and Mitaku S. Identification of G protein-coupled receptor genes from the human genome sequence. *FEBS Lett* 520:97-101 (2002).
- [3] Brink CB, Harvey BH, Bodenstein J, Venter DP and Oliver DW. Recent advances in drug action and therapeutics: relevance of novel concepts in G-protein-coupled receptor and signal transduction pharmacology. *Br J Clin Pharmacol* 57:373-387 (2004).
- [4] Karlin A. On the application of "a plausible model" of allosteric proteins to the receptor for acetylcholine. *J Theor Biol* 16:306-320 (1967).
- [5] Rodbell M, Birnbaumer L, Pohl SL and Krans HM. The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanylnucleotides in glucagon action. *J Biol Chem* 246: 1877-1882 (1971).
- [6] DeLean A, Stadel JM and Lefkowitz RJ. A ternary complex model explains the agonist specific binding properties of the adenylyl cyclase-coupled beta-adrenergic receptor. *J Biol Chem* 255: 7108-7117 (1980).
- [7] Cotecchia S, Exum S, Caron MG and Lefkowitz RJ. Regions of the α_1 -adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. *Proc Natl Acad Sci U S A* 87:2896-2900 (1990).
- [8] Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG and Lefkowitz RJ. Constitutive activation of the α_{1B} -adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. *J Biol Chem* 267:1430-1433 (1992).
- [9] Samama P, Pei G, Costa T, Cotecchia S and Lefkowitz RJ. Negative antagonists promote an inactive conformation of the β_2 -adrenergic receptor. *Mol Pharmacol* 45:390-394 (1994).
- [10] Lefkowitz RJ, Cotecchia S, Samama P and Costa T. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol Sci* 14:303-307 (1993).
- [11] Samama P, Cotecchia S, Costa T and Lefkowitz RJ. A mutation-induced activated state of the β_2 -adrenergic receptor. Extending the ternary complex model. *J Biol Chem* 268:4625-4636 (1993).
- [12] Kenakin T. Agonist-receptor efficacy. I: Mechanisms of efficacy and receptor promiscuity. *Trends Pharmacol Sci* 16:188-192 (1995).
- [13] Yan B, Takahashi T, Johnson R and Spudich JL. Identification of signaling states of a sensory receptor by modulation of lifetimes of stimulus-induced conformations: the case of sensory rhodopsin II. *Biochemistry* 30: 10686-10682 (1991).
- [14] Farahbakhsh ZT, Hideg K and Hubbell WL. Photoactivated conformational changes in rhodopsin: a time-resolved spin label study. *Science* 262: 1416-1419 (1993).
- [15] Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P and Clarke WP. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol* 54:94-104 (1998).
- [16] Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, *et al.* Differential signal transduction by five splice variants of the PACAP receptor. *Nature* 365:170-175 (1993).
- [17] Meller E, Puza T, Diamond J, Lieu HD and Bohmker K. Comparative effects of receptor inactivation, 17 β -estradiol and pertussis toxin on dopaminergic inhibition of prolactin secretion in vitro. *J Pharmacol Exp Ther* 263:462-469 (1992).
- [18] Sagan S, Karoyan P, Chassaing G and Lavielle S. Further delineation of the two binding sites ($R^*(n)$) associated with tachykinin neurokinin-1 receptors using [3-Prolinomethionine(11)]SP analogues. *J Biol Chem* 274:23770-23776 (1999).
- [19] Bonhaus DW, Chang LK, Kwan J and Martin GR. Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. *J Pharmacol Exp Ther* 287:884-888 (1998).
- [20] Krumins AM and Barber R. The stability of the agonist β_2 -adrenergic receptor-Gs complex: evidence for agonist-specific states. *Mol Pharmacol* 52:144-154 (1997).
- [21] Scaramellini C and Leff P. A three-state receptor model: predictions of multiple agonist pharmacology for the same receptor type. *Ann N Y Acad Sci* 861:97-103 (1998).
- [22] Seifert R, Gether U, Wenzel-Seifert K and Kobilka BK. Effects of guanine, inosine, and xanthine nucleotides on $\beta(2)$ -adrenergic receptor/G(s) interactions: evidence for multiple receptor conformations. *Mol Pharmacol* 56:348-358 (1999).
- [23] Abadji V, Lucas-Lenard JM, Chin C and Kendall DA. Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of Gs. *J Neurochem* 72:2032-2038 (1999).
- [24] Barroso S, Richard F, Nicolas-Etheve D, Kitabgi P and Labbe-Jullie C. Constitutive activation of the neurotensin receptor 1 by

- mutation of Phe(358) in Helix seven. *Br J Pharmacol* 135:997-1002 (2002).
- [25] Christopoulos A and Kenakin T. G protein-coupled receptor allostery and complexing. *Pharmacol Rev* 54:323-374 (2002).
- [26] Kenakin T. Drug efficacy at G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 42:349-379 (2002).
- [27] Leff P, Scaramellini C, Law C and McKechnie K. A three-state receptor model of agonist action. *Trends Pharmacol Sci* 18:355-362 (1997).
- [28] Gether U, Lin S and Kobilka BK. Fluorescent labeling of purified β_2 adrenergic receptor. Evidence for ligand-specific conformational changes. *J Biol Chem* 270:28268-28275 (1995).
- [29] Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR, et al. Functionally different agonists induce distinct conformations in the G protein coupling domain of the β_2 adrenergic receptor. *J Biol Chem* 276:24433-24436 (2001).
- [30] Evans PD, Robb S, Cheek TR, Reale V, Hannan FL, Swales LS, et al. Agonist-specific coupling of G-protein-coupled receptors to second-messenger systems. *Prog Brain Res* 106:259-268 (1995).
- [31] Downes GB and Gautam N. The G protein subunit gene families. *Genomics* 62:544-552 (1999).
- [32] Kleuss C, Scherubl H, Hescheler J, Schultz G and Wittig B. Different beta-subunits determine G-protein interaction with transmembrane receptors. *Nature* 358: 424-426 (1992).
- [33] Kleuss C, Scherubl H, Hescheler J, Schultz G and Wittig B. Selectivity in signal transduction determined by gamma subunits of heterotrimeric G proteins. *Science* 259: 832-834 (1993).
- [34] Paasche JD, Attramadal T, Sandberg C, Johansen HK and Attramadal H. Mechanisms of endothelin receptor subtype-specific targeting to distinct intracellular trafficking pathways. *J Biol Chem* 276:34041-34050 (2001).
- [35] Blank JL, Brattain KA and Exton JH. Activation of cytosolic phosphoinositide phospholipase C by G-protein beta gamma subunits. *J Biol Chem* 267:23069-23075 (1992).
- [36] Exton JH. Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* 36:481-509 (1996).
- [37] Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N and Knopf JL. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 65:1043-1051 (1991).
- [38] Schievella AR, Regier MK, Smith WL and Lin LL. Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* 270:30749-30754 (1995).
- [39] Felder CC, Jose PA and Axelrod J. The dopamine-1 agonist, SKF 82526, stimulates phospholipase-C activity independent of adenylyl cyclase. *J Pharmacol Exp Ther* 248:171-175 (1989).
- [40] Jones SB, Halenda SP and Bylund DB. Alpha 2-adrenergic receptor stimulation of phospholipase A2 and of adenylyl cyclase in transfected Chinese hamster ovary cells is mediated by different mechanisms. *Mol Pharmacol* 39:239-245 (1991).
- [41] Allgeier A, Laugwitz KL, Van-Sande J, Schultz G, and Dumont JE. Multiple G-protein coupling of the dog thyrotropin receptor. *Mol Cell Endocrinol* 127:81-90 (1997).
- [42] Brydon L, Roka F, Petit L, de Coppet P, Tissot M, Barrett P, et al. Dual signaling of human Mel1a melatonin receptors via G(i2), G(i3), and G(q/11) proteins. *Mol Endocrinol* 13:2025-2038 (1999).
- [43] Offermanns S, Wieland T., Homann, D, Sandmann J, Bombien E, Spicher K, et al. Transfected muscarinic acetylcholine receptors selectively couple to Gi-type G proteins and Gq/11. *Mol Pharmacol* 45:890-898 (1994).
- [44] Santos-Alvarez J and Sanchez-Margalet V. G protein G alpha q/11 and G alpha i1,2 are activated by pancreastatin receptors in rat liver: studies with GTP-gamma 35S and azido-GTP-alpha-32P. *J Cell Biochem* 73:469-477 (1999).
- [45] Laugwitz KL, Allgeier A, Offermanns S, Spicher K, Van Sande J, Dumont JE, et al. The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. *Proc Natl Acad Sci USA* 93:116-120 (1996).
- [46] Cordeaux Y, Briddon SJ, Megson AE, McDonnell J, Dickenson JM and Hill SJ. Influence of receptor number on functional responses elicited by agonists acting at the human adenosine A(1) receptor: evidence for signaling pathway-dependent changes in agonist potency and relative intrinsic activity. *Mol Pharmacol* 58:1075-1084 (2000).
- [47] Gudermann T, Schoneberg T and Schultz G. Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu Rev Neurosci* 20:399-427 (1997).
- [48] Nasman J, Kukkonen JP, Ammoun S and Akerman KE. Role of G-protein availability in differential signaling by α_2 -adrenoceptors. *Biochem Pharmacol* 62:913-922 (2001).
- [49] Jin LQ, Wang HY and Friedman E. Stimulated D(1) dopamine receptors couple to multiple G α proteins in different brain regions. *J Neurochem* 78:981-990 (2001).
- [50] Chakrabarti S, Prather PL, Yu L, Law PY and Loh HH. Expression of the mu-opioid receptor in CHO cells: ability of μ -opioid ligands to promote α -azidoanilido[32P]GTP labeling of multiple G protein alpha subunits. *J Neurochem* 64:2534-2543 (1995).
- [51] Takasu H, Gardella TJ, Luck MD, Potts JT Jr. and Bringham FR. Amino-terminal modifications of human parathyroid hormone (PTH) selectively alter phospholipase C signaling via the type 1 PTH receptor: implications for design of signal-specific PTH ligands. *Biochemistry* 38:13453-13460 (1999).
- [52] Pommier B, Da Nascimento S, Dumont S, Bellier B, Million E, Garbay C, et al. The cholecystokinin B receptor is coupled to two effector pathways through pertussis toxin-sensitive and -insensitive G proteins. *J Neurochem* 73:281-288 (1999).
- [53] Sagan S, Chassaing G, Pradier L and Lavielle S. Tachykinin peptides affect differently the second messenger pathways after binding to CHO-expressed human NK-1 receptors. *J Pharmacol Exp Ther* 276:1039-1048 (1996).
- [54] Maudsley S, Davidson L, Pawson AJ, Chan R, de Maturana RL and Millar RP. Gonadotropin-releasing hormone (GnRH) antagonists promote proapoptotic signaling in peripheral reproductive tumor cells by activating a G α coupling state of the type I GnRH receptor. *Cancer Res* 64:7533-7544 (2004).
- [55] Gong H, Sun H, Koch WJ, Rau T, Eschenhagen T, Ravens U, et al. Specific $\beta(2)$ AR blocker ICI 118,551 actively decreases contraction through a G(i)-coupled form of the $\beta(2)$ AR in myocytes from failing human heart. *Circulation* 105:2497-2503 (2002).
- [56] Yu Y, Zhang L, Yin X, Sun H, Uhl GR and Wang JB. μ opioid receptor phosphorylation, desensitization, and ligand efficacy. *J Biol Chem* 272:28869-28874 (1997).
- [57] Keith DE, Murray SR, Zaki PA, Chu PC, Lissin DV, Kang L, et al. Morphine activates opioid receptors without causing their rapid internalization. *J Biol Chem* 271:19021-19024 (1996).
- [58] Maudsley S, Gent JP, Findlay JB and Donnelly D. The relationship between the agonist-induced activation and desensitization of the human tachykinin NK2 receptor expressed in *Xenopus* oocytes. *Br J Pharmacol* 124:675-684 (1998).
- [59] Stout BD, Clarke WP and Berg KA. Rapid desensitization of the serotonin(2C) receptor system: effector pathway and agonist dependence. *J Pharmacol Exp Ther* 302:957-962 (2002).
- [60] van Hooft JA and Vijverberg HP. Selection of distinct conformational states of the 5-HT3 receptor by full and partial agonists. *Br J Pharmacol* 117:839-846 (1996).
- [61] Roettger BF, Ghanekar D, Rao R, Toledo C, Yingling J, Pinon D, et al. Antagonist-stimulated internalization of the G protein-coupled cholecystokinin receptor. *Mol Pharmacol* 51:357-362 (1997).
- [62] Kohout TA, Nicholas SL, Perry SJ, Reinhart G, Junger S and Struthers RS. Differential desensitization, receptor phosphorylation, β -arrestin recruitment, and ERK1/2 activation by the two endogenous ligands for the CC chemokine receptor 7. *J Biol Chem* 279:23214-23222 (2004).
- [63] May LT, Avlani VA, Sexton PM and Christopoulos A. Allosteric modulation of G protein-coupled receptors. *Curr Pharm Des* 10:2003-2013 (2004).
- [64] Hebert TE and Bouvier M. Structural and functional aspects of G protein-coupled receptor oligomerization. *Biochem Cell Biol*. 76:1-11 (1998).
- [65] Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, et al. GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* 396:674-679 (1998).
- [66] George SR, Fan T, Xie Z, Tse R, Tam V, Varghese G, et al. Oligomerization of μ - and δ -opioid receptors. Generation of novel functional properties. *J Biol Chem* 275:26128-26135 (2000).
- [67] Franco R, Ferre S, Agnati L, Torvinen M, Gines S, Hillion J, et al. Evidence for adenosine/dopamine receptor interactions: indications

- for heteromerization. *Neuropsychopharmacology* 23:S50-59 (2000).
- [68] Hebert TE, Moffett S, Morello JP, Loisel TP, Bichet DG, Barret C, *et al.* A peptide derived from a β 2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J Biol Chem* 271:16384-16392 (1996).
- [69] Bai M, Trivedi S, Kifor O, Quinn SJ and Brown EM. Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. *Proc Natl Acad Sci USA* 96:2834-2839 (1999).
- [70] Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC and Patel YC. Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem* 275:7862-7869 (2000).
- [71] Ciruela F, Escriche M, Burgueno J, Angulo E, Casado V, Soloviev MM, Canela EI, *et al.* Metabotropic glutamate 1 α and adenosine A1 receptors assemble into functionally interacting complexes. *J Biol Chem* 276:18345-18351 (2001).
- [72] Ferre S, Torvinen M, Antoniou K, Irenius E, Civelli O, Arenas E, *et al.* Adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. *J Biol Chem* 273:4718-4724 (1998).
- [73] Jordan BA and Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* 399:697-700 (1999).
- [74] McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, *et al.* RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* 393:333-339 (1998).
- [75] Evans BN, Rosenblatt MI, Mnayer LO, Oliver KR and Dickerson IM. CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors. *J Biol Chem* 275:31438-31443 (2000).
- [76] Kitamuro T, Takahashi K, Totsune K, Nakayama M, Murakami O, Hida W, *et al.* Differential expression of adrenomedullin and its receptor component, receptor activity modifying protein (RAMP) 2 during hypoxia in cultured human neuroblastoma cells. *Peptides* 11:1795-1801 (2001).
- [77] Sueur S, Pesant M, Rochette L and Connat JL. Antiapoptotic effect of calcitonin gene-related peptide on oxidative stress-induced injury in H9c2 cardiomyocytes via the RAMP1/CRLR complex. *J Mol Cell Cardiol* 39:955-963 (2005).
- [78] Tadokoro K, Nishikimi T, Mori Y, Wang X, Akimoto K, and Matsuoka H. Altered gene expression of adrenomedullin and its receptor system and molecular forms of tissue adrenomedullin in left ventricular hypertrophy induced by malignant hypertension. *Regul Pept* 112:71-78 (2003).
- [79] Freedman NJ and Lefkowitz RJ. Desensitization of G protein-coupled receptors. *Recent Prog Horm Res* 51:319-351 (1996).
- [80] Stoffel RH 3rd, Pitcher JA and Lefkowitz RJ. Targeting G protein-coupled receptor kinases to their receptor substrates. *J Membr Biol* 157:1-8(1997).
- [81] Lawler OA, Miggin SM and Kinsella BT. Protein kinase A-mediated phosphorylation of serine 357 of the mouse prostacyclin receptor regulates its coupling to G(s)-, to G(i)-, and to G(q)-coupled effector signaling. *J Biol Chem* 276:33596-33607 (2001).
- [82] Herrero I, Miras-Portugal MT and Sanchez-Prieto J. Functional switch from facilitation to inhibition in the control of glutamate release by metabotropic glutamate receptors. *J Biol Chem* 273:1951-1958 (1998).
- [83] Luttrell LM. Transmembrane signaling by G protein-coupled receptors. *Meth Mol Biol* In press (2005).
- [84] Pak Y, Pham N and Rotin D. Direct binding of the beta1 adrenergic receptor to the cyclic AMP-dependent guanine nucleotide exchange factor CNrasGEF leads to Ras activation. *Mol Cell Biol* 22:7942-7952 (2002).
- [85] Ali MS, Sayeski PP, Dirksen LB, Hayzer DJ, Marrero MB and Bernstein, KE. Dependence on the motif YIPP for the physical association of Jak2 kinase with the intracellular carboxyl tail of the angiotensin II AT1 receptor. *J Biol Chem* 272:23382-23388 (1997).
- [86] Luttrell LM and Lefkowitz RJ. The role of β -arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci* 115:455-465 (2002).
- [87] Perry SJ and Lefkowitz RJ. Arresting developments in heptahelical receptor signaling and regulation. *Trends Cell Biol* 12:130-138 (2002).
- [88] Luttrell LM, Ferguson SSG, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F-T, *et al.* β -Arrestin-dependent formation of β 2 adrenergic receptor/Src protein kinase complexes. *Science* 283:655-661 (1999).
- [89] DeFea KA, Vaughn ZD, O'Bryan EM, Nishijima D, Dery O and Bunnett NW. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a β -arrestin-dependent scaffolding complex. *Proc Natl Acad Sci USA* 97: 11086-11091 (2000).
- [90] McDonald PH, Chow C-W, Miller WE, LaPorte SA, Field ME, Lin F-T, *et al.* β -Arrestin 2: A receptor-regulated MAPK scaffold for the activation of JNK3. *Science* 290:1574-1577 (2000).
- [91] DeFea KA, Zalevsky J, Thoma MS, Dery O, Mullins RD and Bunnett NW. β -Arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J Cell Biol* 148: 1267-1281 (2000).
- [92] Luttrell LM, Roudabush FL, Choy EW, Miller WE, Field ME, Pierce KL, *et al.* Activation and targeting of extracellular signal-regulated kinases by β -arrestin scaffolds. *Proc Natl Acad Sci USA* 98:2449-2454 (2001).
- [93] Perry SJ, Baillie GS, Kohout TA, McPhee I, Magiera MM, Ang KL, *et al.* Targeting of cyclic AMP degradation to β 2-adrenergic receptors by β -arrestins. *Science* 298:834-836 (2002).
- [94] Gurevich VV, Pals-Rylaarsdam R, Benovic JL, Hosey MM and Onorato JJ. Agonist-receptor-arrestin, an alternative ternary complex with high agonist affinity. *J Biol Chem* 272:28849-28852 (1997).
- [95] Tohgo A, Pierce KL, Choy EW, Lefkowitz RJ and Luttrell LM. β -Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. *J Biol Chem* 277:9429-9436 (2002).
- [96] Bunemann M and Hosey MM. G-protein coupled receptor kinases as modulators of G-protein signaling. *J Physiol* 517:5-23(1999).
- [97] Hayes JS and Brunton LL. Functional compartments in cyclic nucleotide action. *J Cyclic Nucleotide Res* 8:1-16 (1982).
- [98] Buxton IL and Brunton LL. Compartments of cyclic AMP and protein kinase in mammalian cardiomyocytes. *J Biol Chem* 258:10233-10239 (1983).
- [99] Harper JF, Haddox MK, Johanson RA, Hanley RM and Steiner AL. Compartmentation of second messenger action: immunocytochemical and biochemical evidence. *Vitam Horm* 42:197-252 (1985).
- [100] Graesser D and Neubig RR. Compartmentation of receptors and guanine nucleotide-binding proteins in NG108-15 cells: lack of cross-talk in agonist binding among the α 2-adrenergic, muscarinic, and opiate receptors. *Mol Pharmacol* 43:434-443 (1993).
- [101] Xu X, Zeng W, Diaz J and Muallem S. Spatial compartmentalization of Ca^{2+} signaling complexes in pancreatic acini. *J Biol Chem* 271:24684-24690 (1996).
- [102] Steinberg SF and Brunton LL. Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol* 41:751-773 (2001).
- [103] Neubig RR. Membrane organization in G-protein mechanisms. *FASEB J* 8:939-946 (1994).
- [104] Ostrom RS, Post SR and Insel PA. Stoichiometry and compartmentation in G protein-coupled receptor signaling: implications for therapeutic interventions involving G(s). *J Pharmacol Exp Ther* 294:407-412 (2000).
- [105] Galbati F, Razani B and Lisanti MP. Emerging themes in lipid rafts and caveolae. *Cell* 106:403-411 (2001).
- [106] Rimoldi V, Reversi A, Taverna E, Rosa P, Francolini M, Cassoni P, *et al.* Oxytocin receptor elicits different EGFR/MAPK activation patterns depending on its localization in caveolin-1 enriched domains. *Oncogene* 22:6054-6060 (2003).
- [107] Brady AE and Limbird LE. G protein-coupled receptor interacting proteins: emerging roles in localization and signal transduction. *Cell Signal* 14:297-309 (2002).
- [108] Hall RA and Lefkowitz RJ. Regulation of G protein-coupled receptor signaling by scaffold proteins. *Circ Res* 91:672-680 (2002).
- [109] Malbon CC, Tao J and Wang HY. AKAPs (A-kinase anchoring proteins) and molecules that compose their G-protein-coupled receptor signalling complexes. *Biochem J* 379:1-9 (2004).
- [110] Maudsley S, Pierce KL, Zamah AM, Miller WE, Ahn S, Daaka Y, *et al.* The β (2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. *J Biol Chem* 275:9572-9580 (2000).

- [111] Liu G, Shi J, Yang L, Cao L, Park SM, Cui J, *et al.* Assembly of a Ca²⁺-dependent BK channel signaling complex by binding to $\beta 2$ adrenergic receptor. *EMBO J* 23:2196-2205 (2004).
- [112] Bockaert J, Marin P, Dumuis A and Fagni L. The 'magic tail' of G protein-coupled receptors: An anchorage for functional protein networks. *FEBS Lett.* 546:65-72 (2003).
- [113] Hirbec H, Perestenko O, Nishimune A, Meyer G, Nakanishi S, Henley JM, *et al.* The PDZ proteins PICK1, GRIP and syntenin bind multiple glutamate receptor subtypes. Analysis of PDZ binding motifs. *J Biol Chem* 277: 15221-15224 (2002).
- [114] Sala C, Piech V, Wilson NR, Passafaro M, Liu G and Sheng M. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* 31: 115-130 (2001).
- [115] Mao L, Yang L, Tang SY, Samdani S, Zhang G and Wang JQ. The scaffold protein Homer 1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascade in neurons. *J Neurosci* 25: 2741-2752 (2005).
- [116] Salanova M, Priori G, Barone V, Intravaia E, Flucher B, Ciruela F, McIlhinney RA, Parys JB, *et al.* Homer protein and InsP(3) receptors co-localise in the longitudinal sarcoplasmic reticulum of skeletal muscle fibres. *Cell Calcium* 32: 193-200 (2002).
- [117] Westhoff JH, Hwang SY, Scott Duncan R, Ozawa F, Volpe P, Inokuchi K, *et al.* Vesl/homer proteins regulate ryanodine receptor type 2 function and intracellular calcium signaling. *Cell Calcium* 34: 261-269 (2003).
- [118] Fagni L, Worley PF and Ango F. Homer as both a scaffold and transduction molecule. *Sci STKE* 137:RE8 (2002)
- [119] Bermak JC, Li M, Bullock C and Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. *Nat Cell Biol* 3: 492-498 (2001).
- [120] Prezeau L, Richman JG, Edwards SW and Limbird LE. The zeta isoform of 14-3-3 proteins interact with the third intracellular loop of different $\alpha 2$ -adrenergic receptor subtypes. *J Biol Chem* 274: 13462-13469 (1999).
- [121] Couve A, Kittler JT, Uren JM, Calver AR, Pangalos MN, Walsh FS, *et al.* Association of GABA(B) receptors and members of the 14-3-3 family of signaling proteins. *Mol Cell Neurosci* 17: 317-328 (2001).
- [122] Hebert LE, Scherr PA, Bienias JL, Bennett DA and Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol.* 60:1119-22 (2003).
- [123] Coyle JT, Price DL and DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 219:1184-1190 (1983).
- [124] Ladner CJ and Lee JM. Pharmacological drug treatment of Alzheimer disease: the cholinergic hypothesis revisited. *J Neuropathol Exp Neurol* 57:719-731 (1998).
- [125] Waller SB, Bal MJ, Reynolds MA and London ED. Muscarinic binding and choline acetyltransferase in postmortem brains of demented patients. *Can. J. Neurol. Sci.* 13:528-532 (1986).
- [126] Cohen RM, Podruchny TA, Bokde ALW, Carson RE, Herscovitch P, Keisewetter DO, *et al.* Higher in vivo muscarinic-2 receptor distribution volumes in aging subjects with an apolipoprotein E-4 allele. *Synapse* 49:150-156 (2003).
- [127] Hellstrom-Lindahl E. Modulation of beta-amyloid precursor protein processing and tau phosphorylation by acetylcholine receptors. *Eur J Pharmacol* 393: 255-263 (2000).
- [128] Zuchner T, Schliebs R and Perez-Polo JR. Down-regulation of muscarinic acetylcholine receptor M2 adversely affects the expression of Alzheimer's disease-relevant genes and proteins. *J Neurochem.* 95, 20-32 (2005).
- [129] Farias GG, Godoy JA, Hernandez F, Avila J, Fisher A and Inestrosa NC. M1 muscarinic receptor activation protects neurons from beta-amyloid toxicity. A role for Wnt signaling pathway. *Neurobiol Dis* 17: 337-348 (2004).
- [130] Andersen MB, Fink-Jensen A, Peacock L, Gerlach J, Bymaster F, Lundbaek JA, *et al.* The muscarinic M1/M4 receptor agonist xanomeline exhibits antipsychotic-like activity in Cebus apella monkeys. *Neuropsychopharmacology* 28: 1168-1175 (2003).
- [131] Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, *et al.* International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* 54: 247-264 (2002).
- [132] Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S, *et al.* SGS742: the first GABAB receptor in clinical trials. *Biochem Pharmacol* 68:1479-1487 (2004)..
- [133] Shiozaki K and Iseki E. Decrease in GTP-sensitive high affinity agonist binding of muscarinic acetylcholine receptors in autopsied brains of dementia with Lewy Bodies and Alzheimer's disease. *J Neurol Sci* 223:145-148 (2004).
- [134] Muma NA, Mariyappa R, Williams K and Lee JM. Differences in regional and subcellular localization of Gq/11 and RGS4 protein levels in Alzheimer's disease: correlation with muscarinic M1 receptor binding parameters. *Synapse* 47:58-65 (2003)
- [135] Popescu BO, Cedazo-Minguez A, Benedikz E, Nishimura T, Winblad B, Ankarcrana M, *et al.* γ -secretase activity of presenilin 1 regulates acetylcholine muscarinic receptor-mediated signal transduction *J Biol Chem* 279:6455-6464 (2004).
- [136] Suo Z, Wu M, Citron BA, Wong GT and Festoff BW. Abnormality of G protein coupled receptor kinase at prodromal and early stages of Alzheimer's disease: An association with early β -amyloid accumulation. *J Neurosci* 24:3444-3452 (2004).
- [137] Suo Z, Wu M, Citron BA, Palazzo RE and Festoff BW. Rapid tau aggregation and delayed hippocampal neuronal death by persistent thrombin signaling. *J Biol Chem* 278:37681-37689 (2003).
- [138] Donovan FM and Cunningham DD. Signaling pathways involved in thrombin-induced cell protection. *J Biol Chem* 273:12746-12752 (1998).
- [139] Rocchi A, Micheli D, Ceravolo R, Manca ML, Tognoni G, Siciliano G, *et al.* Serotonergic polymorphisms (5HTLPR and 5HT2A): association studies with psychosis in Alzheimers disease. *Genet Test* 7:309-314 (2003).
- [140] Feuerstein TJ, Gleichauf O and Landwehrmeyer GB. Modulation of cortical acetylcholine release by serotonin: the role of substance P interneurons. *Naunyn-Schmeidebergs Arch. Pharmacol.* 354:618-626 (1996).
- [141] Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT and Ramirez MJ. Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. *Neuropsychopharmacology* 29: 410-416 (2004).
- [142] Perez-Garcia GS and Meneses A. Effects of the potential 5-HT7 receptor agonist AS 19 in an autoshaping learning task. *Behav Brain Res* 163: 136-140 (2005).
- [143] Maillet M, Robert SJ and Lezoualc'h F. New insights into serotonin 5-HT4 receptors : a novel therapeutic target for Alzheimer's disease? *Curr Alzheimer Res* 1: 97-85 (2004).
- [144] Schechter LE, Smith DL, Rosenzweig-Lipson S, Sukoff SJ, Dawson LA, Marquis K, *et al.* Lecozotan (SRA-333): A selective serotonin 1 A receptor antagonist that enhances the stimulated release of glutamate and acetylcholine in the hippocampus and possesses cognitive-enhancing properties. *J. Pharmacol. Exp. Ther.* 314:1274-1289 (2005).
- [145] Dewar D, Chalmers DT, Graham DI and McCulloch J. Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: an autoradiographic study of the hippocampus. *Brain Res.* 553: 58-64 (1991).
- [146] Vincent AM, Mohammad Y, Ahmad I, Greenberg R and Maiese K. Metabotropic glutamate receptors prevent nitric oxide induced programmed cell death. *J. Neurosci. Res.* 50: 549-564 (1997).
- [147] Lee RK, Jimenez J, Cox AJ and Wurtman RJ. Metabotropic glutamate receptors regulate APP processing in hippocampal neurons and cortical astrocytes derived from fetal rats. *Ann N Y Acad Sci* 777: 338-343 (1996).
- [148] Furukawa K, Barger SW, Blalock EM and Mattson MP. Activation of K⁺ channels and suppression of neuronal activity by secreted beta-amyloid precursor protein. *Nature* 379: 74-78 (1996).
- [149] Taylor DL, Diemel LT and Pocock JM. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. *J Neurosci* 23: 2150-2160 (2003).
- [150] Riedel G, Opitz T and Reymann KG. Blockade of metabotropic glutamate receptors protects hippocampal neurons from hypoxia-induced cell death in rat in vivo. *Prog. Neuro-psychopharmacol. Biol. Psychiatry* 20: 1253-1263 (1996).
- [151] Moldrich RX and Beart PM. Emerging signaling and protein interactions mediated via metabotropic glutamate receptors. *Curr Drug Targets CNS Neurol Disord* 2:109-122 (2003).
- [152] Higgins GA, Ballard TM, Kew JNC, Grayson-Richards J, Kemp JA, Adam G, *et al.* Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. *Neuropharmacology* 46:907-917 (2004).

- [153] May LT, Avlan VA, Sexton PA and Christopoulos A. Allosteric modulation of G protein-coupled receptors. *Curr. Pharm. Design* 10:2003-2013 (2004).
- [154] Blake AD, Bot G, Freeman JC and Reisine T. Differential opioid agonist regulation of the mouse mu opioid receptor. *J Biol Chem* 272:782-790 (1997).
- [155] Amara A, Gall SL, Schwartz O, Salamero J, Montes M, Loetscher P, *et al.* HIV coreceptor downregulation as antiviral principle: SDF-1 α -dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J Exp Med* 186: 139-146 (1997).
- [156] Signoret N, Oldridge J, Pelchen-Matthews A, Klase PJ, Tran T, Brass LF, *et al.* Phorbol esters and SDF-1 induce rapid endocytosis and down-regulation of the chemokine receptor CXCR4. *J Cell Biol* 139:651-664 (1997).
- [157] Simmons G, Clapham PR, Picard L, Offord RE, Rosenkilde MM, Schwartz TW, *et al.* Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* 276:276-279 (1997).
- [158] Mack M, Luckow B, Nelson PJ, Cihak J, Simmons G, Clapham PR, *et al.* Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J Exp Med* 187:1215-1224 (1998).
- [159] Domingo E, Menendez-Arias L, Quinones-Mateu ME, Holguin A, Gutierrez-Rivas M, Martinez MA, *et al.* Viral quasispecies and the problem of vaccine-escape and drug-resistant mutants. *Prog Drug Res* 48:99-128 (1997).
- [160] Harrigan PR, Bloor S and Larder BA. Relative replicative fitness of zidovudine-resistant human immunodeficiency virus type 1 isolates *in vitro*. *J Virol* 72:3773-3778 (1998).
- [161] Schols D, Este JA, Cabrera C and De Clercq E. T-cell-line-tropic human immunodeficiency virus type 1 that is made resistant to stromal cell-derived factor 1 α contains mutations in the envelope gp120 but does not show a switch in coreceptor use. *J Virol* 72:4032-4037 (1998).
- [162] Rodriguez-Frade JM, Vila-Coro AJ, Martin A, Nieto M, Sanchez-Madrid F, Proudfoot AE, *et al.* Similarities and differences in RANTES- and (AOP)-RANTES-triggered signals: implications for chemotaxis. *J Cell Biol* 144:755-765 (1999).
- [163] Kuhmann SE, Platt EJ, Kozak SL and Kabat D. Cooperation of multiple CCR5 coreceptors is required for infections by human immunodeficiency virus type 1. *J Virol* 74:7005-7015 (2000).
- [164] Mukhopadhyay S, McIntosh HH, Houston DB and Howlett AC. The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. *Mol Pharmacol* 57:162-170 (2000).
- [165] Romano C, Yang WL and O'Malley KL. Metabotropic glutamate receptor 5 is a disulfide-linked dimer. *J Biol Chem* 271:28612-28616 (1996).
- [166] White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, *et al.* Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* 396:679-682 (1998).
- [167] Ciruela F, Saura C, Canela EI, Mallol J, Lluís C and Franco R. Ligand-induced phosphorylation, clustering, and desensitization of A1 adenosine receptors. *Mol Pharmacol* 52:788-797 (1997).
- [168] Cvejic S and Devi LA. Dimerization of the delta opioid receptor: implication for a role in receptor internalization. *J Biol Chem* 272:26959-26964 (1997).
- [169] Bai M, Trivedi S and Brown EM. Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J Biol Chem* 273:23605-23610 (1998).
- [170] Kenakin TP. Agonist selective receptor states: the new level of selectivity? *Pharmacology News* 5:20-25 (1998).
- [171] Lefkowitz RJ, Pierce KL and Luttrell LM. Dancing with different partners: protein kinase phosphorylation of seven membrane-spanning receptors regulates their G protein-coupling specificity. *Mol Pharmacol* 62:971-974 (2002).
- [172] El-Bakri NK, Adem A, Suliman IA, Mulugeta E, Karlsson E, Lindgren JU, *et al.* Estrogen and progesterone treatment: effects on muscarinic M(4) receptor subtype in the rat brain. *Brain Res.* 6:131-137 (2002).
- [173] Mulutega E, Karlsson E, Islam A, Kalaria R, Mangat H, Winblad B, *et al.* Loss of muscarinic M4 receptors in hippocampus of Alzheimer patients. *Brain Res* 960:259-262 (2003).
- [174] Savaskan E, Hock C, Olivieri G, Bruttel S, Rosenberg C, Hulette C, *et al.* Cortical alterations of angiotensin converting enzyme, angiotensin II and AT1 receptor in Alzheimer's dementia. *Neurobiol Aging* 22:541-546 (2001).
- [175] Kalaria RN and Andorn AC. Adrenergic receptors in aging and Alzheimer's disease: decreased alpha 2-receptors demonstrated by [3H]p-aminoclonidine binding in prefrontal cortex. *Neurobiol Aging* 12:131-136 (1991).
- [176] Kemppainen N, Laine M, Laakso MP, Kaasinen V, Nagren K, Vahlberg T, *et al.* Hippocampal dopamine D2 receptors correlate with memory functions in Alzheimer's disease. *Eur J Neurosci* 18: 149-154 (2003).
- [177] Jansen KL, Faull RL, Dragunow M, Synek BL. Alzheimer's disease: changes in hippocampal N-methyl-D-aspartate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors--an autoradiographic study. *Neuroscience* 39:613-627 (1990).
- [178] Kumar U. Expression of somatostatin receptor subtypes (SSTR1-5) in Alzheimer's disease brain: an immunohistochemical analysis. *Neuroscience* 134:525-538 (2005).
- [179] Chu DC, Penney JB Jr and Young AB. Quantitative autoradiography of hippocampal GABAB and GABAA receptor changes in Alzheimer's disease. *Neurosci Lett* 82: 246-252 (1987).
- [180] Barreda-Gomez G, Giralt MT and Rodriguez-Puertas R. Effects of central galanin administration on muscarinic cholinergic and galanin receptor G protein coupling. *Neuropeptides* 39:157-160 (2005).
- [181] Chan-Palay V. Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. *J. Comp. Neurol.* 273:543-557 (1988).
- [182] Silva, AP, Xapelli S, Grouzmann E and Cavadas C. The putative neuroprotective role of neuropeptide Y in the central nervous system. *Curr. Drug Targets CNS Neurol. Disord.* 4:331-347 (2005).
- [183] Savaskan E, Ayoub MA, Ravid R, Angeloni D, Fraschini F, Meier F, *et al.* Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. *J Pineal Res* 38:10-16 (2004).
- [184] Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M and de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* 25: 1904-1913 (2005).
- [185] Higuchi M, Yanai K, Okamura N, Meguro K, Arai H, Itoh M, *et al.* Histamine H(1) receptors in patients with Alzheimer's disease assessed by positron emission tomography. *Neuroscience* 99:721-729 (2000).
- [186] Kohzuki M, Onodera H, Yasujima M, Itoyama Y, Kanazawa M, Sato T, *et al.* Endothelin receptors in ischemic rat brain and Alzheimer brain. *J. Cardiovasc. Pharmacol.* 26:329-331 (1995).
- [187] Bajetto A, Bonavia R, Barbero S, Florio T and Schettini G. Chemokines and their receptors in the central nervous system. *Front Neuroendocrinol.* 22:147-184 (2001).