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Heteromerization of G Protein-Coupled Receptors: Relevance to Neurological Disorders and Neurotherapeutics

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Abstract

Because G protein-coupled receptors (GPCRs) are numerous, widely expressed and involved in major physiological responses, they represent a relevant therapeutic target for drug discovery, particularly regarding pharmacological treatments of neurological disorders. Among the biological phenomena regulating receptor function, GPCR heteromerization is an important emerging area of interest and investigation. There is increasing evidence showing that heteromerization contributes to the pharmacological heterogeneity of GPCRs by modulating receptor ontogeny, activation and recycling. Although in many cases the physiological relevance of receptor heteromerization has not been fully established, the unique pharmacological and functional properties of heteromers are likely to lead to new strategies in clinical medicine. This review describes the main GPCR heteromers and their implications for major neurological disorders such as Parkinson's disease, schizophrenia and addiction. A better understanding of molecular mechanisms underlying drug interactions related to the targeting of receptor heteromers could provide more specific and efficient therapeutic agents for the treatment of brain diseases.

Keywords

Heteromerization; heteromer; GPCR; neurological disorder; drug discovery

INTRODUCTION

G protein-coupled receptors (GPCRs) are involved in most physiological responses, and alterations of their function are related to many pathological states such as hypertension, diabetes, pain, asthma, immunological and neurological disorders. 30% to 40% of current pharmaceutical drugs target GPCRs and the regulation of their function is essential for therapeutic strategies. GPCR heteromerization refers to the direct interaction between at least two different functional receptors forming a complex with specific biochemical and functional properties different from those of its component receptor units [1,2]. Heteromerization is emerging as increasingly important in creating functional receptor diversity. Diverse studies using biochemical, pharmacological, functional or behavioural approaches indicate that GPCRs can exist as dimeric entities [3–13]. While nearly all

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domains of GPCRs have been identified as involved in interaction within specific heteromers, the majority of studies implicate the transmembrane helices and intracellular domains as the major interaction interfaces [14–18]. The stochiometry of these complexes is not completely established. Future investigation will clarify the various structures and composition of these novel receptor complexes [2]. We focus here on current evidence for GPCR heteromers.

Prior to the recent evidence for receptor heteromers, experimental data relevant to functional communication between GPCRs following their ligand activation were attributed to crosstalk of distinct signaling pathways downstream of GPCRs. The universality of this classical interpretation of experimental results has been brought into question with the recent discovery that different GPCRs can interact directly. Thus some GPCR crosstalk mechanisms may occur directly at the level of the receptor complex. Consonant with this possibility, many studies have revealed functional consequences of GPCR heteromerization on each step of the life cycle of a receptor, from its targeting to the regulation of its pharmacological, signaling and internalization properties (Fig. 1).

Heterocomplex formation has been found to contribute to receptor maturation, folding and expression at the cell surface by modulating its targeting or internalization [19]. The chemokine receptor 1 (CXCR1)-chemokine receptor 2 (CXCR2) heteromer is an example of the role of receptor heteromerization in their targeting to the plasma membrane [20]. For β_2-adrenergic receptor (β_2AR)-delta opioid receptor (DOR) [21], mu opioid receptor (MOR)-somatostatin 2A receptor ($SSTR_{2A}$) [22], α_{2A}-adrenergic receptor (α_{2A}R)-β_1-adrenergic receptor (β_1AR) [23] or α_{1A}-α_{1B}-adrenergic receptor [24] heteromers, activation of one receptor is sufficient to induce the internalization of both receptors. For DOR-kappa opioid receptor (KOR) [1], β_2AR-KOR [21], β_1AR-β_2AR [25] or β_2AR-β_3-adrenergic receptor (β_3AR) [26] heteromers, activation of one receptor prevents the internalization of the other one. Others studies show that GPCR heteromerization can modify receptor internalization by determining the interaction with β–arrestin [27].

Once at the cell surface, the crosstalk between GPCRs can lead to specific receptor pharmacological profiles [28]. Indeed, negative ($SSTR_{2A}$-somatostatin $SSTR_3$ [29], LH/CGR-TSHR [30] or chemokine CCR_2-CCR_5 [6]) and positive (β_1AR-β_2AR [31]) cooperative binding processes have been described for many heteromers [32,33]. GPCR heteromerization has other important functional roles. Heteromers can regulate the receptor-activated signal transduction positively (D_2R-somatostatin $SSTR_5$ [34], angiotensin II type 1 receptor-bradykinin B_2 receptor [35], α_{1B}-α_{1D}-adrenergic receptors [36] or α_{2C}-adrenergic receptor-β_2AR [37]) or negatively ($SSTR_{2A}$-$SSTR_3$ [29] or melatonin MT1 receptor-orphan GPR50 receptor [38]). In some cases, the heteromerization can change the selectivity of a GPCR for different types of G protein or β-arrestin, leading to the regulation of a new signaling pathway [39]. This occurs for DOR-MOR [40,41], CCR_2-CCR_5 [15] and D_1R-D_2R [42,43] heteromers.

GPCR heteromers introduce new properties leading to a large complexity of receptor-mediated regulatory responses. The combinatorial complexity introduced by this phenomenon of heterocomplex formation makes it more difficult to understand and to study the biological mechanisms of GPCRs. The existence of functional GPCR heteromers represents both a major challenge and an opportunity for neuroscience and drug discovery. The number of GPCRs for which evidence suggests a functional role of heteromerization is rapidly increasing. Given the large number of GPCR genes, their ability to form combinations raises the daunting possibility that there are tens or even hundreds of thousands of unique receptor heteromers in the brain and nervous system. Elucidating the function of each of these potential distinct receptor complexes represents a considerable

challenge. On the other hand, this proliferation of functional complexes brings with it a potential for developing drugs targeted to specific heteromers that have greatly increased therapeutic specificity and reduced side-effects. Because GPCRs include many important therapeutic targets, a better understanding of GPCR heteromerization is essential to understand the pharmacology of potential drugs, especially in the treatment of neurological disorders [10,11,14,44]. In this review, we examine salient examples of GPCR crosstalk mechanisms implicated in the pathophysiology of the central nervous system, with particular emphasis on functions that are more likely to result from their heteromeric association (Table 1). We also consider new therapeutic approaches targeting specifically these complexes.

ADENOSINE AND DOPAMINE RECEPTOR HETEROMERS

Adenosine (A_1R-$A_{2A}R$) and Dopamine (D_1R-D_2R; D_1R-D_3R; D_2R-D_3R) Receptor Heteromers

Adenosine and dopamine receptors are implicated in many neurological processes (motivation, pleasure, cognition, memory, learning, motor control) [45] and alterations of their signaling are involved in several neuropsychiatric disorders, including Parkinson's disease [46,47], schizophrenia [48], drug addiction [49,50] and Alzheimer's disease [51]. Adenosine A_1R and $A_{2A}R$ and dopamine D_1R and D_2R are major targets for neurologic drugs (antipsychotics or psychostimulants) and investigations of their interaction mechanisms are particularly relevant for the understanding of their functional crosstalk and the development of new therapeutic strategies.

The first evidence for adenosine and dopamine receptor dimerization has been provided for adenosine A_1R, dopamine D_1R and D_2R homomers [52–54]. The colocalization of these different receptor subtypes in the same areas led to the study of their possible heteromerization. Recently, A_1R and $A_{2A}R$ heteromeric complexes have been identified in living cells and striatal nerve terminals by combining BRET (Bioluminescence Resonance Energy Transfer), FRET (Fluorescence Resonance Energy Transfer) and co-immunoprecipitation approaches [55]. Both D_1R and D_2R can also form heteromers and these complexes present specific signaling and internalization properties that are distinct from that of D_1R and D_2R homomers [42]. The D_1R-D_2R heteromer is associated to the coupling of $G_{q/11}$ protein and activates the phopholipase C cascade in the striatum, while D_1R is specifically coupled to G_s protein and D_2R to $G_{i/o}$ protein [43]. Also D_1R-D_2R heteromers cointernalize after selective activation of either receptor [56]. Recently, D_1R has been demonstrated to interact in living cells with another dopamine receptor subtype, D_3R [57,58]. Binding assays on striatal membrane preparations show modifications of receptor pharmacological properties, since D_3R activation increases D_1R agonist affinity. Moreover, behavioural effects mediated by D_1R are enhanced when D_3R is activated suggesting the presence of D_1R-D_3R heteromerization in striatum [57]. D_2R-D_3R heteromers detected in living cells and the receptor co-localization in striatopallidal gamma-aminobutyric acid (GABA) neurons are also potentially relevant to the pathophysiology and treatment of both Parkinson's disease and schizophrenia [59–61].

Due to the adenosine-dopamine antagonistic functional crosstalk in the central nervous system and the striatal co-localization of their different GPCR subtypes, the existence of functional complexes between them has been investigated. Indeed, adenosine and dopamine systems modulate activity of striatonigral and striatopallidal pathway neurons. The dopaminergic D_2R neurons express both A_1R and $A_{2A}R$ while the dopaminergic D_1R neurons express only A_1R. Based on co-immunoprecipitation, BRET and FRET experiments, several groups have provided evidence for specific A_1R-D_1R and $A_{2A}R$-D_2R heteromers both in living cells and striatum [62–67].

Adenosine A_1R-Dopamine D_1R Heteromer

Co-immunoprecipitation experiments performed on fibroblast cells co-transfected with two receptors provide evidence for a specific A_1R-D_1R but not A_1R-D_2R heteromeric complex that is modulated by ligands. Pretreatment of cells with a selective A_1R agonist increases the amount of A_1R-D_1R complex detected, whereas a D_1R agonist has the opposite effect [67].

In brain, adenosine and dopamine antagonize each other's pharmacological and biochemical effects; this may result in part from their activity at the A_1R-D_1R heteromer complex [45,62]. Pharmacological experiments show that adenosine A_1R agonists shift the dopamine D_1R binding state from high-affinity to low-affinity. A_1R expressed in presynaptic dopaminergic nerve terminals decreases dopamine release by inhibiting the D_1R activation. A_1R antagonists lead to a potentiation in the D_1R-induced cyclic adenosine monophosphate (cAMP) response [68], an effect that could result from their regulation of G proteins having offsetting activities. D_1R is predominantly coupled to G_s protein which stimulates adenylate cyclase and A_1R to $G_{i/o}$ proteins which have inhibitory effects. However, the heteromer introduces crosstalk effects that cannot be entirely attributed to the separate signaling effects of the two receptors. Coactivation of the A_1R-D_1R heteromer induces a decrease of the affinity of D_1R for agonist and D_1R-induced cAMP accumulation. A recent report demonstrates that adenosine A_1R activation enhances dopamine D_1R desensitization in human embryonic kidney 293 (HEK293) cells and this effect is blocked by A_1R antagonists [69]. Another study shows that activation of A_1R blocks D_1R desensitization in COS-7 cells [70]. These differences also highlight the importance of the cellular context; HEK293 and COS7 cells do not express the same regulator proteins such as G protein-coupled receptor kinases (GRK) and β-arrestin protein that regulate D_1R desensitization. Overall, these data suggest strongly that A_1R-D_1R heteromers alter receptor ligand binding, signaling and desensitization mechanisms [69–73]. At the neuronal level, double immunofluorescence assays demonstrate a colocalization of adenosine A_1R and dopamine D_1R in soma and dendrites of cortical neurons [74,75]. Many behavioral studies report functional antagonism of adenosine A_1R-dopamine D_1R. Selective adenosine agonists inhibit the motor activating effects induced by dopamine agonists while adenosine antagonists enhance the same effect [45]. Moreover, the last descriptions of pharmacological properties of the A_1R-D_1R heteromer provide a basis for the design of new pharmaceutical compounds to treat addiction. Interestingly, it was reported that cocaine, a potent stimulant of the central nervous system, targets the adenosine-dopamine heteromeric complex in rat nucleus accumbens by inhibiting the physical interaction between A_1R and D_1R [50].

Adenosine $A_{2A}R$-Dopamine D_2R Heteromer

In the early 1990s, evidence for adenosine $A_{2A}R$-dopamine D_2R heteromers in striatum was first described. The antagonistic receptor crosstalk found in the central nervous system leads to the consideration of $A_{2A}R$ antagonists as neuroprotective molecules and antiparkinsonian compounds [76,77]. $A_{2A}R$-D_2R heteromer existence was subequently confirmed by co-immunoprecipitation [63,78] and BRET / FRET methods [66,79,80] in living cells and striatum. Heteromerization between these receptors was described as constitutive since this phenomenon is $A_{2A}R$ and D_2R agonist-independent. Moreover, it was found by using D_1-D_2 chimeric receptors that the fifth transmembrane domain and particularly an Arg-rich epitope of the N-terminus of the third intracellular loop of D_2R may constitute interfaces that interact with a phosphorylated Ser-rich epitope of the C-terminal part of $A_{2A}R$ in the heteromer [18,65].

The observation that activation of the $A_{2A}R$ leads to a decrease of D_2R agonist binding site affinity suggests a close and functional association between $A_{2A}R$ and D_2R [66,76,77]. However, which components of antagonist signaling between the two receptors occur at the

level of the receptor heteromer or at the level of downstream signaling is not known. Some opposing effects of $A_{2A}R$ and D_2R agonists may also take place downstream of the receptor since the receptors are coupled to G proteins than can have opposing effects, G_s protein and $G_{i/o}$ protein, respectively. However, activation of the $A_{2A}R$ decreases coupling of the D_2R to its $G_{i/o}$ protein [81]. Furthermore, stimulation of D_2R decreases the coupling of $A_{2A}R$ to its G_s protein [63,82]. Thus the $A_{2A}R$-D_2R heteromer affects receptor binding, G protein coupling and may be also involved in the cross-desensitization mechanisms *via* agonist-induced coaggregation and cointernalization of both receptors [45,63].

In the central nervous system, the striatonigral and the striatopallidal GABAergic neurons represent more than 90% of the striatal neuronal population. A_1R-D_1R interactions would be expected to modulate the function of striatonigral neurons. $A_{2A}R$-D_2R interactions would alter the function of striatopallidal neurons. Both populations play crucial roles in the pathophysiology of basal ganglia disorders [45]. Numerous studies have suggested that the $A_{2A}R$-D_2R heteromer in the central nervous system may provide a new therapeutic target for treating Parkinson's disease, schizophrenia and addiction [45,81,83,84]. In animal models, adenosine agonists and antagonists are potent atypical neuroleptics and antiparkinsonian drugs, respectively [45,85–87]. Behavorial studies show that selective A_2R agonists inhibit the motor activating effects induced by D_2R agonists, while A_2R antagonists enhance the same effects promoting motor inhibition [45]. This enhancement of dopamine D_2R transmission by adenosine A_2R antagonists help reverse the motor impairment observed in models of Parkinson’s disease (reduced D_2R signaling). Thus A_2R antagonists have antiparkinsonian activity [88–91]. On the other hand, the striatopallidal GABAergic neurons, which contain $A_{2A}R$-D_2R heteromers, are also known to be targets for antipsychotic drugs, mainly D_2R antagonists. An A_2R agonist, CGS 21680, shows antipsychotic activity in both amphetamine and phencyclidine rodent models of schizophrenia [87,92]. The antipsychotic activity of A_2R agonists may be mediated through the inhibition of the D_2R within the A_2R-D_2R heteromer [87,92]. Moreover, it was found that an adenosine uptake inhibitor enhances the antipsychotic effects of a dopamine receptor antagonist, supporting the role of the dopaminergic transmission regulation in the treatment of schizophrenia [93]. Lastly, A_2R agonists have also been suggested as cocaine addiction drugs by altering receptor signaling within striatal $A_{2A}R$-D_2R heteromers [84].

Adenosine $A_{2A}R$-Dopamine D_2R-Glutamate mGluR5 Heteromer

In addition to adenosine and dopamine transmission, glutamate transmission plays an important role in the function of striatal GABAergic efferent neurons originating in the nucleus accumbens. Adenosine A_2R and dopamine D_2R also colocalize with metabotropic glutamate mGlu5 receptor (mGluR5) in striatum. A_2R and mGluR5 are co-expressed in nearly half of striatal glutamatergic nerve terminals. An association of A_2R and mGluR5 has been suggested by co-immunoprecipitation studies [94–96]. Pharmacological studies suggest an allosteric interaction in the D_2R-mGluR5 heteromer; mGluR5 agonists decrease the affinity of the striatal D_2R agonist binding sites [97,98]. The A_2R, D_2R and mGluR5 heterocomplex has been speculated in striatum [83]. Consonant with this idea, A_2R and mGluR5 agonists synergistically increase ventral pallidal extracellular level of GABA in the nucleus accumbens leading to a potential stability of the inhibitory dopaminergic D_2R effects on the striato-pallidal GABA pathway [99]. In Parkinson’s disease, glutamate transmission is overactive due to the reduced inhibitory D_2R effect. It has been reported that the treatment of Parkinsons leads to regulation of mGluR5 and A_2R activation [100,101]. Indeed, mGluR5 and A_2R antagonists, which are known to be antiparkinsonian, could act synergistically by targeting the proposed A_2R-D_2R-mGluR5 heterocomplex [102]. It has been reported that mGluR5 antagonists induce their motor activator effects only when both A_2R and D_2R are co-expressed [101]. Furthermore, the intramembrane A_2R-D_2R interaction

is positively regulated by mGluR5 activation [94,97,98,103]. These results suggest that A_2R and mGluR5 antagonists may have their antiparkinsonian effects in the glutamatergic synapses of the dorsal striato-pallidal GABA neurons by enhancing the dopamine D_2R signaling through their A_2R-D_2R-mGluR5 heteromerization [102]. Therefore, a simultaneous combination between A_2R and mGluR5 antagonists enhancing their efficacy in reversing Parkinsonian motor deficits may constitute a novel nondopaminergic therapy by avoiding the adverse chronic effects of dopaminergic drugs [100,104,105]. A role of the striatal A_2R-mGluR5 interactions in addiction has also been proposed, which may partly explain the modulation of cocaine and methamphetamine dependence by mGluR5 agonists [103,106]. Thus, the A_2R-D_2R-mGluR5 heteromer could have a role in controlling the ventral striato-pallidal GABA neurons involved in drug addiction and schizophrenia and provide an interesting potential target for future therapies.

Adenosine $A_{2A}R$-Dopamine D_2R-Cannabinoid CB_1R Heteromer

Cannabinoid represents an important inhibitory neuromodulator acting in the central nervous system. The activation of cannabinoid CB_1 receptors (CB_1R) leads to motor depression. CB_1R are co-expressed predominantly with D_2R in the soma and dendrites of the ventral striato-pallidal GABA neurons and with A_2R in corticostriatal glutamate terminals [107–112]. Recently, co-immunoprecipitation and BRET experiments showed that CB_1R and A_2R interact together in living cells and striatum and that this interaction is functional since it mediates the cannabinoid motor effects. Biochemical experiments in the neuroblastoma cell line and behavioral tests in mice indicated that striatal CB_1R activation-induced synaptic effects depend on A_2R activation [113]. Indeed, A_2R antagonists lead to an inhibition of CB_1R agonist-induced motor depressant effects [114]. A reduction of alterations in the motor depression effects induced by CB_1R agonists in A_2R knockout mice was also described [115]. Therefore, the A_2R-CB_1R heteromer may mediate the motor depressant effects of CB_1R agonists. Moreover, evidence for CB_1R-D_2R heteromers in cell lines and striatum has been provided by co-immunoprecipitation experiments, FRET experiments and pharmacological analysis indicating the reduction of the affinity of D_2R agonist binding by CB_1R agonists and the agonist-induced enhancement of CB_1R-D_2R heteromerization. Several studies have reported antagonistic interactions between CB_1R and D_2R activation, as well as the antiparkinsonian actions of CB_1R antagonists [116–118].

CB_1R knockout mice show an increase in their baseline levels on anxiety [119]. Furthermore, CB_1R activation mediates central effects and more particularly the addictive properties of cannabinoids such as tetrahydrocannabinol (THC), the main psychoactive ingredient of marihuana [120]. In view of the potential role of CB_1R-D_2R heteromer and A_2R antagonists as potential therapeutic agents in drug dependence [121–123], the antagonistic CB_1R-D_2R interactions may also involve the A_2R [124–126]. A_2R may play a role in the behavioral inhibition exerted by the CB_1R agonist on D_2R agonist-induced locomotor hyperactivity. In support of this view, striatal A_2R is known to directly interact with both D_2R [66] and CB_1R [114], which, as noted above, may suggest the formation of a A_2R-CB_1R-D_2R multisubunit complex in brain. Furthermore, A_2R-CB_1R-D_2R heteromers have been recently identified in living cells by using a method combining bimolecular fluorescence complementation and BRET techniques [127–129].

In post-mortem brain of schizophrenics, pharmacological studies showed an alteration of A_2R [130], D_2R [131] and CB_1R [132] expression. In animal models, antipsychotic treatment leads to a down-regulation of CB_1R expression in nucleus accumbens [133] which could represent an adaptative mechanism that reduces the endocannabinoid-mediated suppression of GABA release [134]. Taking these results together, the A_2R-CB_1R-D_2R heteromer may also have a role in schizophrenia and represents a potential target for antipsychotic compounds.

SEROTONIN 5-HT_{2A}R-GLUTAMATE mGluR2 HETEROMER

The function of serotonin in brain is strongly associated with specific physiological responses, ranging from modulation of neuronal activity and transmitter release to behavioural changes [135]. Glutamate serves as the principal neurotransmitter of the pyramidal cells, which are the sources of efferent and interconnecting pathways of the cerebral cortex and limbic systems. A physical and functional interaction between serotonin 5-HT_{2A} receptor and metabotropic glutamate subtype 2 receptor (mGluR2) has been identified in cortical pyramidal neurons [136–140]. This heteromer constitutes a new and uncommon example of a physical association between GPCRs of different classes since mostly heteromers which have been described involve GPCRs belonging to the same class. Fluorescent *in situ* hybridization (FISH) experiments showed the co-localization of 5-HT_{2A}R and mGluR2 mRNA expression in layer V of mouse somatosensory cortex and mouse cortical primary cultures. Evidence for a receptor association has been provided by co-immunoprecipitation, BRET, FRET, binding assays in heterologous systems and brain cortex. The glutamate mGluR2-serotonin 5-HT_{2A}R heteromer represents an example of GPCR association having specific consequences on the pharmacology, signaling and behavioural pharmacology of drugs acting at the receptor components [136]. Competition binding experiments showed that a mGluR2 agonist increases the affinity of hallucinogenic drugs for the 5-HT_{2A}R-binding site, and a 5-HT_{2A}R agonist decreases the affinity of agonists for the mGluR2 binding site (Fig. 2). Changes in high affinity binding caused by mGluR2-5-HT_{2A}R crosstalk suggest that this complex may serve to integrate serotonin and glutamate receptor signaling and modulate G-protein coupling. Pharmacologically, similar hallucinogenic and non-hallucinogenic serotonin 5-HT_{2A}R agonists elicit qualitatively different downstream signaling events as reflected in the pattern of gene transcription they induce [141]. More precisely, hallucinogenic 5-HT_{2A}R agonists induce specific receptor conformational changes within the mGluR2-5-HT_{2A}R heteromer leading to the recruitment of specific cortical 5-HT_{2A}R mediated signaling pathways [142]. Hallucinogenic 5-HT_{2A}R agonists induce through the heteromerization with mGluR2 the activation of both $G\alpha_{q/11}$ and also $G\alpha_{i/o}$ proteins, while 5-HT_{2A}R and mGluR2 alone are coupled to $G\alpha_{q/11}$ and $G\alpha_{i/o}$ proteins, respectively (Fig. 2). Similar evidence for specification of G-protein subtype regulation was also observed for the endogeneous mGluR2-5-HT_{2A}R complex with membranes from cortical primary cultures [136]. Interestingly, the mGluR2-5-HT_{2A}R heteromer may constitute thus a new target for antipsychotic drugs in the treatment of schizophrenia since mGluR2 agonists inhibit *via* allosteric interactions within the heteromer the 5-HT_{2A}R-mediated hallucinogen-specific $G\alpha_{i/o}$ signaling [143–145].

The differences in the capacity of the mGluR2 and mGluR3 to change the pharmacological properties of 5-HT_{2A}R and their close sequence similarity were the basis to identify the specific mGluR2 domains responsible for heteromer formation. The study of a series of molecular chimeras of the mGluR2 and mGluR3 demonstrated that the segment containing transmembrane helices 4 and 5 of mGluR2 is necessary and sufficient for the heteromeric formation with 5-HT_{2A}R [136]. These results provide structural evidence for the formation of a heteromer between members of different classes of GPCRs and identify the involvement of specific protein domains at the interface of a GPCR heteromer.

The expression of each component of the mGluR2-5-HT_{2A}R heteromer was studied in the brain of patients with schizophrenia. The receptor densities in cortical membranes of untreated schizophrenic subjects were significantly altered, showing increased 5-HT_{2A}R and decreased mGluR2 expression levels [136]. It is possible that this dysregulation of 5-HT_{2A}R and/or mGluR2 expression may alter cortical integration of serotonin and glutamate signaling and contribute to the abnormalities of thought and behavior in schizophrenia. These results are consistent with the hypothesis that the mGluR2-5-HT_{2A}R heteromer

integrates serotonin and glutamate signaling to regulate the sensory gating functions of the cortex, a process that is disrupted in psychosis [137–140]. This complex warrants further study of its possible role in the symptoms of schizophrenia and of its potential as a therapeutic target.

ADRENERGIC α_{2A}R-OPIOID MOR HETEROMER

Another example of receptor association is the α_{2A}R-MOR heteromer. α_{2A}R and MOR are both members of GPCR class A that couple to the same $G_{i/o}$ class of G proteins. These receptors affect the nociceptive system and are particularly involved in depression of neurotransmitter release in the spinal cord [146–150]. Activation of MOR by agonists such as morphine results in strong antinociceptive effects [151,152]. *In vivo*, evidence for a colocalization of α_{2A}R and MOR was provided by immunocytochemical experiments in hippocampal neurons and in neurons of of the medial nucleus tractus solitarius, suggesting a crosstalk between the two systems [153,154].

The first evidence supporting an association between the two receptors comes from studies using mice without functional α_{2A}R. These mice show a decrease in the analgesic potency of spinally administered morphine compared with wild-type mice. These results are consonant with the effects depending on an interaction between α_{2A}R and MOR [150]. A direct interaction between α_{2A}R and MOR has been demonstrated using biophysical, biochemical and pharmacological studies in transfected cells and primary neurons [154–156]. Treatment with either MOR or α_{2A}R agonist increases the quantity of immunoprecipitable receptor complex. In addition, in transfect cells, expression of both receptors increases level of G protein activation and mitogen-activated protein kinase (MAPK) phosphorylation induced with either agonist. These results suggest that the α_{2A}R-MOR complex increases the signaling and efficacy of specific MOR (morphine) or α_{2A}R (clonidine) agonists [154]. The α_{2A}R-MOR heteromer may play a role in the adrenergicopioid crosstalk seen *in vivo* in older studies, such as the potentiating effect of clonidine on morphine analgesia [157,158]. While the link between the functional crosstalk of the two receptors and their physical interaction is controversial [155], recently, an approach based on FRET confirmed the existence of the α_{2A}R-MOR heteromer and its effects on cell signaling [156]. Indeed, cross-conformational changes within the heteromer lead to a transinhibition of receptor activation [156]. Using MOR or α_{2A}R agonists in electrophysiology experiments measuring voltage-gated Ca2+, a mutual cross-desensitization between MOR and α_{2A}R-mediated current inhibition was demonstrated. This effect is closely associated with simultaneous internalization of MOR and α_{2A}R. Furthermore, inhibition of p38 MAPK prevented the crossdesensitization as well as cointernalization of MOR and α_{2A}R. Changes in receptor trafficking profiles suggested that p38 MAPK activity was required for initiating MOR internalization and maintaining possible MOR and α_{2A}R association during their cointernalization. Moreover, it was demonstrated the p38 MAPK and β-arrestin 2 dependent cross-regulation between neuronal MOR and α_{2A}R [159].

Biochemical assays in postmortem human brain also suggest that the α_{2A}R-MOR heteromer might play a role in opioid addiction. Adaptive changes of α_{2A}R density in brain have been demonstrated in animal models of opioid dependence [160,161], as well as in postmortem human brain of opioid addicts [162]. It has also been suggested that, in cortical brain membranes from chronic abusers of opioids, the MOR-G protein coupling is unchanged, whereas the potency of the selective α_{2A}R agonist UK14304 to stimulate [^{35}S]GTPγS binding decreased [163]. Concurrently with results *in vitro* in tissue cultures, these findings in postmortem human brain of opioid addicts raise the possibility that α_{2A}R down-regulation and desensitization may alter the signaling properties of the α_{2A}R-MOR heteromer and therefore prevent the down-regulation of MOR and their functional uncoupling for G

proteins *in vivo*. Further experiments are required in MOR and/or α_{2A}R knock-out mouse models to test this hypothesis.

In summary, these results are consistent with the notion that the physical interaction between MOR and α_{2A}R plays an important role in modulating their signaling. These effects may result from agonist-induced changes in receptor conformation and/or heteromer association.

OTHER GPCR HETEROMERS

Given the large number of GPCRs, it is likely that many other heteromers exist and contribute to normal brain function and disease pathogenesis. Evidence for the existence of various other GPCR heteromers has been reported. A functional interaction between adenosine A_1R and metabotropic glutamate mGluR1α has been shown in transfected cells as well in cerebellar and primary cortical neurons and may constitute a possible therapeutic target for antipsychotic drugs [164]. In addition, the adenosine A_1R could also interact with serotonin 5-HT_{2A}R, since an A_1R agonist abolishes the molecular and behavioural effects induced by a hallucinogenic 5-HT_{2A}R agonist while a A_1R antagonist produces opposite effects [165,166]. Both A_1R and mGluR2 couple to the $G_{i/o}$ protein and their agonists have somewhat similar effects on the hallucinogen-induced 5-HT_{2A}R activation.

Recently, opposing pharmacological and behavioral properties have been described for the dopamine D_2R and histamine H_3 receptor (H_3R) in striatum. These results are consistent with an interaction between the two GPCRs, D_2R-H_3R heteromers have been identified by BRET in transfected cells [167]. It is conceivable that a complex can exist between A_{2A}R-D_2R-H_3R *in vivo* since these three GPCR are co-expressed in striato-pallidal GABA neurons. In addition to adenosine A_{2A}R antagonists and dopamine D_2R agonists, histamine H_3R antagonists may represent novel antiparkinsonian agents suitable for targeting the heteromer.

Other GPCR heteromers have been implicated in neuropsychiatric disorders. Vasopressin V_{1b} receptor (V_{1b}R) and the receptor of bird ortholog vasopressin (VT2R) heteromerize with corticotropin releasing hormone receptor type 1 (CRHR1). These two heteromers have recently been identified and correlated with altered receptor signaling [168–170]. Due to the co-localization of these receptors in corticotrope pituitary cells and the respective role of each in the stress axis, these heteromeric complexes are interesting candidates for playing a role in mood disorder or depression.

GPCR HETEROMERS AND DRUG DISCOVERY

GPCR heteromeric complexes having unique biochemical and functional properties provide important new targets for drug discovery [44,171–173]. While the identification of heteromers has complicated the search for drugs by providing a combinatorial increase in the number of targets, this complexity brings with it a new possibility for increased therapeutic efficacy and reduced side effects. Receptor heteromerization represents a direct mechanism for crosstalk between two extracellular signals. The many emerging examples of modification of the pharmacological and signaling properties of a GPCR in presence of another one highlights the importance of identifying appropriate heteromeric complexes as drug targets. The potential roles of opioid receptor heteromers in analgesia and drug tolerance/dependence [174–177] and of serotonin/glutamate heteromers in the effects of antipsychotics agents [143] suggest that heteromers will be increasingly important as drug targets for diseases of the brain and nervous system.

Presently, most ligands acting at heteromers are molecules that target preferentially one of the receptors components of the heteromer (Fig. 3). For example, SKF83959 which has

antiparkinsonian effects, is believed to act at the D_1R-D_2R heteromer and induce $G_{q/11}$ protein signaling in the brain. SKF83959 binds preferentially to the D_1R component of the complex [43,178]. It is likely that a number of GPCR ligands having therapeutic potential (D_2R, $A_{2A}R$, 5-$HT_{2A}R$, mGluR2, mGluR5, CB_1R, and H_3R ligands) exert some of their clinical effects in diseases ranging from Parkinson's to schizophrenia from their activity at GPCR heteromers. Furthermore, some dopamine receptor ligands originally described as being selective for a single receptor may be heteromer-specific. An example is the antipsychotic l-stepholidine, which is pharmacologically a D_1R agonist and a D_2R antagonist, that may act specifically at D_1R-D_2R heteromers [179]. In the same manner, antiparkinsonian agents such as S32504, ropinirole and pramipexole also represent selective agonists for D_2R-D_3R heteromers [59]. Thus, while heteromer-specific compounds are more difficult to characterize, their use could reduce side effects in the treatment of neurological diseases.

A different approach is to develop bivalent ligands binding both individual components of the heteromer [180] (Fig. 3). These compounds contain two different GPCR ligands (two agonists, two antagonists or one of each) that are linked through amino acid spacers [181]. A recent study describes the development of new adenosine $A_{2A}R$ antagonist – dopamine D_2R agonist bivalent ligands useful for the detection of $A_{2A}R$-D_2R heteromers [182]. In view of selective $A_{2A}R$ antagonists and D_2R agonists as antiparkinsonian compounds, the adenosine / dopamine bivalent action could be used for the treatment of Parkinson's disease by improving specificity and efficacy. In addition, GPCR ligands that act as allosteric modulators represent another potential therapeutic tool to target receptor heteromeric entities [8,28,183].

CONCLUSIONS

Experimental results on GPCR heteromers highlight the difficulty in identifying a causal link between functional crosstalk of two receptor systems and physical association of the receptor components in a complex. Determining factors regulating the receptor crosstalk mechanisms involved in neurological disorders is an increasingly important avenue for developing improved drugs. Before the "dimerization" era, GPCR crosstalk was attributed entirely to the integrative effects of distinct signaling pathways coupled to each receptor. As described above, GPCR dimerization is a likely mechanism for the interactive effects of drugs as well as of the responses to individual drugs [19,184]. However, the role of heteromers in signaling crosstalk has only been demonsrated conclusively in a few instances and crosstalk resulting from the regulation of downstream signaling molecules will remain an important mechanism for interactive effects [185].

Until recently, most experiments demonstrating a direct GPCR interaction and its functional consequences have been performed in artificial cell systems using modified receptors. Nevertheless, some examples by using functional (knockout mice), biochemical (co-immunoprecipitation) and pharmacological (allosteric modulations) approaches strongly support the existence of functional heteromers *in vivo* [5,6,31,35,136,164,186–191]. However, with the exception of positive cooperative binding [13,192,193], most results from such studies do not exclude mechanisms independent of direct receptor interaction. Techniques suitable for studying direct receptor-receptor interactions in native tissues require new reagents, including receptor / heteromer-specific antibodies, heteromer-specific ligands, ligands containing fluorophore labelling and approaches to study the functional effects of receptor complexes in specific subcellular compartments *in vivo* [194–196]. One new approach is the development of a biophysical method to detect physical GPCR interactions in native tissues using time-resolved FRET and selective fluorescent ligands

[197,198]. The relationship of altered receptor heteromerization and symptoms of brain disease remains to be determined.

We have focused our attention on direct GPCR interactions with putative implications in neuropsychiatric disorders (Table 1). The allosteric interaction in the receptor heteromer namely the intermolecular interaction by which binding of a ligand to one of the receptor units in the heteromer changes the binding properties of another receptor unit constitutes the predominant biochemical effect [2,199,200]. Allosteric mechanisms within heteromers induce facilitating or inhibitory effects on ligand binding and also alter receptor signaling selectivity and trafficking [27,43]. GPCR heteromerization leads to a diversity of pharmacological profiles and considerably increases the repertoire of GPCR functional responses. This diversity is increased if GPCRs exist as complexes comprising than two sub-units [2]. Indeed, recent studies report the existence of oligomers (or multimers) [128,183,201–205]. The stochiometry of the receptor complexes and the functional consequences of complex formation are areas of intense current research.

The direct interactions between GPCRs can also depend on partner proteins at the extracellular, intramembrane or intracellular compartments [14,199,200]. Interactions between heteromers and various partners such as G proteins, β-arrestins, calmodulin, regulators of G protein signaling (RGS) or receptor activity modifying proteins (RAMP) have consequences in the regulation of signal transduction [206,207]. Another challenge is determining the stoichiometry of interaction between heteromers and their signaling / adapter proteins. Studies in this area are likely to yield additional targets for the design of specific drugs for brain disease.

GPCR heteromerization provides promising insights into GPCR pathophysiology and the development of novel heteromer-targeted drugs. Recent studies have demonstrated incontrovertible existence for functional GPCR heteromers in a native context. The characterization of heteromerization represents a challenge and an opportunity for developing new drugs for brain disease with increased therapeutic efficacy and reduced side effects.

ABBREVIATIONS

GPCR	G protein-coupled receptor
A_1R	Adenosine A_1 receptor
$A_{2A}R$	Adenosine A_{2A} receptor
D_1R	Dopamine D_1 receptor
D_2R	Dopamine D_2 receptor
D_3R	Dopamine D_3 receptor
CB_1R	Cannabinoid 1 receptor
mGluR5	Metabotropic glutamate receptor 5
mGluR2	Metabotropic glutamate receptor 2
5-$HT_{2A}R$	Serotonin 2A receptor
$\alpha_{2A}R$	α_{2A}-adrenergic receptor
β_1AR	β_1-adrenergic receptor
β_2AR	β_2-adrenergic receptor

MOR	Mu opioid receptor
DOR	Delta opioid receptor
$SSTR_{2A}$	Somatostatin 2A receptor
H_3R	Histamine H_3 receptor
BRET	Bioluminescence resonance energy transfer
FRET	Fluorescence resonance energy transfer
GABA	Gamma-aminobutyric acid
MAPK	Mitogen-activated protein kinase

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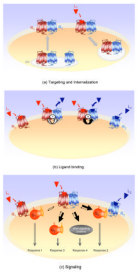

Fig. (1). Roles of GPCR heteromerization

(**a**) Heteromerization is first involved on maturation, folding and receptor expression at the cell surface by modulating its targeting from the endoplasmic reticulum or internalization. (**b**) At the plasma membrane, GPCR heteromerization can induce positive (+) or negative (−) cooperative ligand binding leading to specific pharmacological profiles. (**c**) Heteromerization can have a functional role by promoting or attenuating the specific G-protein coupling of a single receptor constituting the heteromer. In some cases, this regulation can open to a new signaling pathway (to a new G protein coupling or to a switch to other signaling protein recruitment like β-arrestin recruitment). *(R1: receptor 1, R2: receptor 2, L1: ligand 1, L2: ligand 2, G1: G protein 1, G2: G protein 2, G3: G protein 3, ER: endoplasmic reticulum)*

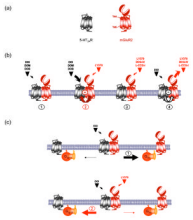

Fig. (2). Functional crosstalk between glutamate mGluR2 and serotonin 5-HT$_{2A}$R resulting of their heteromerization

(**a**) The physical interaction between the serotonin 5-HT$_{2A}$R and glutamate mGluR2 belonging to the GPCR classes 1 and 3, respectively, is mediated by mGluR2 tramsmembrane domains 4 (TM4) and 5 (TM5). (**b**) 5-HT$_{2A}$R hallucinogen agonist (DOI / DOM / DOB) affinities are higher in the presence of the mGluR2 agonist (LY379268) which induces a positive cooperative binding within the heteromer (1 and 2). By contrast, mGluR2 agonist (LY379268 / DCG-IV / L-CCG-I) affinities are lower in the presence of the 5-HT$_{2A}$R agonist (DOI) which induces a negative cooperative binding within the heteromer (3 and 4). **(c)** While 5-HT$_{2A}$R and mGluR2 are mainly coupled to the G$_{q/11}$ and G$_{i/o}$ proteins, respectively, hallucinogen (DOI)-stimulated mGluR2-5-HT$_{2A}$R heteromer enhances significantly the G$_{i/o}$ protein coupling (1). This effect is reversed in the presence of the mGluR2 agonist (LY379268) which enhances the G$_{q/11}$ protein coupling (2) suggesting a putative role of this compound as an antipsychotic drug able to abolish specific hallucinogen-induced effects. See [136].

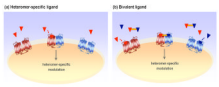

Fig. (3). GPCR heteromer-specific drug targets

(**a**) Specific targeting of a GPCR only involved in a heteromeric formation. Regarding receptor heteromers involved in neurological diseases, antiparkinsonian SKF83959 [178] and antipsychotic l-stepholidine [179] are specific for D_1R-D_2R heteromer while antiparkinsonians S32504, ropinirole and pramipexole are specific for D_2R-D_3R heteromer [59]. (**b**) Bivalent ligands modulate specifically heteromers by binding both GPCRs. The adenosine $A_{2A}R$ antagonist – dopamine D_2R agonist bivalent ligand specific for the $A_{2A}R$-D_2R heteromer could be used as a potent antiparkinsonian agent [182].

Table 1

Examples of GPCR Heteromers with Implications in Neurological Disorders

Heteromer	Receptor co-localization	Receptor association (methods)	Functional Relevance	Clinical Relevance	Heteromer-Specific Therapeutic Drugs	References
A_1R-$A_{2A}R$	Striatum	Cell lines, striatum *(IP, B, F)*	Pharmacology	Drug Tolerance		[55]
D_1R-D_2R	Striatum	Cell lines, striatum *(IP, F)*	Signaling, internalization	PD, Schizophrenia	I-stepholidine (antipsychotic) SKF83959 (antiparkinsonian)	[42,43,56,178, 179]
D_1R-D3R	Striatum	Cell lines *(B, F, PA)*	Pharmacology, behavior	PD		[57,58]
D_2R-D_3R	Striato-pallidal GABA neurons	Cell lines, striatum *(IP)*	Pharmacology, signaling	PD, Schizophrenia	S32504, pramipexole, ropinirole (antiparkinsonians)	[59–61]
A_1R-D_1R	Cortex, striatum	Cell lines, striatum *(IP)*	Pharmacology, signaling, internalization	Schizophrenia, PD, addiction		[45,50,62,64, 67–75]
$A_{2A}R$-D_2R	Striato-pallidal GABA neurons	Cell lines, striatum *(IP, B, F, PA)*	Pharmacology, signaling, internalization	Schizophrenia, PD, addiction	$A_{2A}R$ antagonist / D_2R agonist bivalent ligand (antiparkinsonian)	[18,45,63,65, 66,76–84, 88–93,182]
$A_{2A}R$-mGluR5	Striatal glutamatergic terminals	Cell lines, striatum *(IP)*	Signaling	PD, addiction		[94–96,103, 106]
D_2R-mGluR5	striatum	Cell lines, striatum *(PA)*	Pharmacology	Schizophrenia		[97,98]
$A_{2A}R$-D_2R-mGluR5	Striato-pallidal GABA neurons	Cell lines, striatum *(IP, S)*	Signaling	Schizophrenia, PD, addiction		[83,94,97–105]
$A_{2A}R$-CB_1R	Cortico-striatal glutamate terminals	Cell lines, striatum *(IP, B)*	Signaling, behavior	nd		[113–115]
CB_1R-D_2R	Striato-pallidal GABA neurons	Cell lines, striatum *(IP, F, PA)*	Pharmacology, signaling, behavior	Schizophrenia, PD, addiction		[107,116–118, 122–126]
$A_{2A}R$-CB_1R-D_2R	Striatum	Cell lines *(S)*	nd	Schizophrenia		[124–129,134]
mGluR2-5-$HT_{2A}R$	Prefrontal cortex	Cell lines, cortex *(IP, B, F, PA)*	Pharmacology, signaling, behavior	Schizophrenia		[136–145]
$\alpha_{2A}R$-MOR	Medial nucleus tractus solitarius, hippocampal neurons	Cell lines *(IP, B, F)*	Signaling	Addiction		[150,153–163]
A_1R-mGluR1α	Cortex	Cell lines, cerebellum *(IP)*	Signaling	Schizophrenia		[164]
A_1R-5-$HT_{2A}R$	Prefrontal cortex	nd	Signaling, behavior	Schizophrenia		[165,166]
D_2R-H_3R	Striato-pallidal GABA neurons	Cell lines, striatum *(B, PA)*	Pharmacology, behavior	PD		[167]
CRHR1-$V_{1b}R$(VT2R)	Corticotrop cells	Cell lines *(IP, B, F)*	Signaling	Mood disorder, depression		[168–170]

Receptor associations have been detected in heterologous systems (cell lines) and brain except for the A_1R-mGluR1α heteromer detected in cerebellar synaptosomes *(IP: co-immunoprecipitation, B: Bioluminescence Resonance Energy Transfer, F: Fluorescence Resonance Energy Transfer, S: Sequential Resonance Energy Transfer, PA: pharmacological allosteric effects detected by binding assays, PD: Parkinson's disease, nd: not determined).*