Send Orders for Reprints to reprints@benthamscience.net

422 *Current Neuropharmacology,* **2019**, *17,* **422-437**

REVIEW ARTICLE

Functional Cross-Talk between Adenosine and Metabotropic Glutamate Receptors

David Agustín León-Navarro[1,2], José Luis Albasanz[1,2,3] and Mairena Martín[1,2,3,*]

[1]*Departamento de Química Inorgánica, Orgánica y Bioquímica. CRIB, Universidad de Castilla-La Mancha;* [2]*Facultad de Ciencias y Tecnologías Químicas, Avenida Camilo José Cela, 10, 13071 Ciudad Real;* [3]*Facultad de Medicina de Ciudad Real, Camino Moledores s/n. 13071 Ciudad Real, Spain*

ARTICLE HISTORY

Received: November 08, 2017
Revised: March 19, 2018
Accepted: April 13, 2018

DOI:
10.2174/1570159X16666180416093717

Abstract: G-protein coupled receptors are transmembrane proteins widely expressed in cells and their transduction pathways are mediated by controlling second messenger levels through different G-protein interactions. Many of these receptors have been described as involved in the physiopathology of neurodegenerative diseases and even considered as potential targets for the design of novel therapeutic strategies. Endogenous and synthetic allosteric and orthosteric selective ligands are able to modulate GPCRs at both gene and protein expression levels and can also modify their physiological function. GPCRs that coexist in the same cells can homo- and heteromerize, therefore, modulating their function. Adenosine receptors are GPCRs which stimulate or inhibit adenylyl cyclase activity through G_i/G_s protein and are involved in the control of neurotransmitter release as glutamate. In turn, metabotropic glutamate receptors are also GPCRs which inhibit adenylyl cyclase or stimulate phospholipase C activities through G_i or G_q proteins, respectively. In recent years, evidence of crosstalk mechanisms between different GPCRs have been described. The aim of the present review was to summarize the described mechanisms of interaction and crosstalking between adenosine and metabotropic glutamate receptors, mainly of group I, in both *in vitro* and *in vivo* systems, and their possible use for the design of novel ligands for the treatment of neurodegenerative diseases.

Keywords: Metabotropic glutamate receptors, adenosine receptors, crosstalk, oligomerization, protein interaction, signalling.

1. INTRODUCTION

Cellular communication is a key feature of evolution. The multitude of stimuli that can reach a cell may elicit different cellular responses according to the signalling pathways present in the cell. In addition, there may exist a cross-communication between these signalling pathways which is crucial for the fine-tuning of cellular response. The first interaction or crosstalk process can take place at the surface receptor level [1]. The crosstalk between two given pathways can modify not only the response to their corresponding specific signals (ligands) but also their regulation (*e.g*. desensitization). This crosstalk can be only functional (no direct contact exists between signalling components) or can involve a direct association, mainly at the receptor level through di/oligomerization [2]. G protein-coupled receptors (GPCRs) transduce a diversity of extracellular signals and regulate virtually every aspect of physiology, however, their overstimulation can be harmful, resulting in cellular toxicity or uncontrolled cellular growth. Therefore, different *desensitization* (decrease in response to repeated or continuous stimulation) mechanisms have been developed to avoid such overstimulation, involving a network of kinases, ubiquitin ligases, and adaptor proteins acting on GPCRs [3]. GPCR-mediated transduction pathways can be modulated by the interaction between different receptors. In fact, oligomerization exerts a significant impact on receptor function and physiology which contributes to increasing the diversification of receptor signalling, pharmacology, regulation, crosstalk, internalization and trafficking [4, 5].

GPCR signalling can be modulated not only by ligand binding to orthosteric sites of receptors but also by binding to allosteric sites, which are involved in the affinity and selectivity of ligands for GPCRs and represent a promising target for the design of new drugs to treat disorders of the central nervous system through GPCR activation/blockade [6]. Moreover, the formation of hetero-oligomers may provide novel allosteric binding pockets for ligand binding and new targeting possibilities [7].

Adenosine is an endogenous purinergic nucleoside present in all cells where forms part of the nucleic acids and the energy currency of life, ATP. Furthermore, adenosine is also

*Address correspondence to this author at the Facultad de Ciencias y Tecnologías Químicas. Universidad de Castilla-La Mancha, Avenida Camilo José Cela, 10. 13071 Ciudad Real; Tel: +34926295300 ext, 6603;
E-mail: mairena.martin@uclm.es

1570-159X/19 $58.00+.00

able to act as signalling molecule through binding adenosine receptors (ARs) which belong to class A GPCR superfamily being linked, therefore, to different transduction mechanisms including adenylate cyclase (AC), phospholipase C (PLC), phospholipase A_2, phospholipase D and G protein-coupled inwardly-rectifying potassium channels (GIRK). ARs have been further subdivided into four main types (A_1, A_{2A}, A_{2B} and A_3) according to pharmacological profile, functional coupling to adenylyl cyclase activity and tissue distribution. Thus, A_1 and A_3 receptors inhibit AC through $G_{i/o}$ proteins whereas A_{2A} and A_{2B} receptors stimulate AC through G_s proteins. ARs are widely expressed throughout the body where they participate in physiological processes such as immune function, heart rate and circulation, lipolysis, sleep regulation, and angiogenesis [8]. GPCRs dimerization is also an important research field in adenosine receptors [9, 10] and can be involved in physiological and pathological processes by homo- and heteromerization processes. Thus, adenosine receptors can interact with each other and with different GPCRs such as A_1/A_{2A}, A_{2A}/CB_1, $A_{2A}/CB_1/D_2$, $A_{2A}/mGlu_5$, or A_{2A}/D_2 [4]. This heterodimerization can be modulated by purines and xanthines such as caffeine and theophylline [11]. Even A_1/A_{2A}, A_{2A}/D_2, $A_{2A}/mGlu_5$ heterodimers and $A_{2A}/mGlu_5/D_2$ heterotrimers have been related to Alzheimer disease [12, 13].

L-glutamate is a naturally occurring aminoacid widely known to be the main excitatory neurotransmitter in the Vertebrate Central Nervous System although its actions reach peripheral and non-neural tissues such as adrenal gland, pancreas, immune cells and a large etcetera [14]. Glutamate actions are mediated by a group of membrane receptors named glutamate receptors which include metabotropic (mGlu) and ionotropic (iGlu) glutamate receptors. MGlu receptors also belong to class C GPCR superfamily and have been subclassified into three groups: Group I ($mGlu_1$ and $mGlu_5$) which stimulate PLC activity through a $G_{q/11}$ protein, Group II ($mGlu_2$ and $mGlu_3$) and Group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) which inhibit AC activity through a $G_{i/o}$ protein [15]. iGlu receptors are ligand-gated ion channels involved in fast excitatory neurotransmission and which include AMPA, NMDA and Kainate receptors according to the response to agonist.

One of the most relevant functions of adenosine in Nervous System is to regulate synaptic transmission. This neuromodulatory action is possible thanks to a combination of effects at presynaptic and postsynaptic levels that permits adenosine to influence both the release of several neurotransmitters, including L-glutamate, and also regulate neurotransmitter action [16]. The neuromodulatory role of adenosine is mainly linked to A_1 and A_{2A} receptors since both are predominant in brain. In fact, A_1 receptor is the most abundant and widespread in the brain whereas A_{2A} receptors are concentrated in the basal ganglia although they can also be found throughout the brain at lower densities. Concerning synaptic localization, both receptors are mainly distributed at presynaptic regions but they have been also detected at postsynaptic membranes [17]. Thus, ultrastructural analysis has demonstrated the A_{2A} receptors postsynaptic localization in the dendrites and dendritic spines of GABAergic neurons in rat striatum [18]. In contrast, hippocampal A_{2A} receptors are mostly located in nerve terminals and particularly in the presynaptic active zone [19]. Striatal A_{2A} immunoreactivity has been observed primarily at glutamatergic synapses, predominantly at the postsynaptic but also at the presynaptic site [18, 20]. This localization of A_{2A} receptors is in line with its suggested role in the fine-tuning modulation of glutamatergic neurotransmission in striatal GABAergic neurons both at the postsynaptic and presynaptic level [18, 20-21]. Moreover, A_{2A}, $mGlu_5$ and D_2 receptors do oligomerize in GABAergic striatopallidal neurons, and these receptors co-distributed in postsynaptic structures along the extrasynaptic and perisynaptic plasma membrane of spines, establishing asymmetrical, putative glutamatergic, synapses with axon terminals [22]. Thus, although striatal A_{2A} receptors are mostly located postsynaptically, they are also present in the presynaptic active zone. Conversely, although A_{2A} receptors are mostly located presynaptically in the hippocampus, they are also present in the postsynaptic density. Accordingly, A_{2A} receptors are able to modulate the release of neurotransmitters (GABA, acetylcholine and glutamate) in the striatum, whereas they are able to control postsynaptic responsiveness in the hippocampus [19]. This pre- and postsynaptic presence A_{2A} receptors has been also reported in monkey basal ganglia, with a high degree of colocalization between A_{2A} and $mGlu_5$ in dendrites and spines of striatal neurons where they functionally interact [23].

The excitatory neurotransmitter L-glutamate can also act as neuromodulator through binding mGlu receptors which are widely distributed throughout the CNS where they specifically localize in both synaptic and extrasynaptic regions. In general, group I-mGlu receptors are frequently found at postsynaptic regions, but also located presynaptically [24, 25], and their activation often increases neuronal excitability whereas group II and group III mGlu receptors are mainly distributed on presynaptic terminals where they can inhibit neurotransmitter release at both inhibitory (GABAergic) and excitatory (glutamatergic) synapses [26, 27].

2. FUNCTIONAL INTERACTION BETWEEN ADENOSINE AND MGLU RECEPTORS

In recent decades, the functional interaction between adenosine and metabotropic glutamate receptors has received a great deal of attention in the GPCR field. Most of these studies have been carried out in cells from Central Nervous System using both *in vivo* and *in vitro* approaches. The results of these studies have indicated that the *crosstalk* between the two groups of receptors took place at multiple levels including second messenger production, synaptic transmission, neurotransmitter release and behavioural effects. In the present review, we will focus on group I mGlu /adenosine receptor interactions, although there are also data showing the functional interaction between group II/III mGlu and adenosine receptors [28, 29].

2.1. Adenosine A_1 and Metabotropic Glutamate Receptors Cross-talk

Ogata and coworkers showed the first evidence of a crosstalk process between both groups of receptors in 1994 using cultured type 1 astrocyte from the hippocampus. They observed that the activation of A_1 receptor potentiated group I

mGlu receptor-mediated calcium responses. Two years later, the same research group also described the opposite crosstalk showing that the accumulation of cAMP induced by the non-selective adenosine receptor agonist, 2-chloroadenosine, was potentiated by different agonists of group I mGlu receptors [28, 30]. The utilization of more selective receptor ligands allowed the study of the functional interaction with more precision. Thus, Toms and Roberts (1999) [31], by using DHPG, a selective group I mGlu receptor agonist, described how A_1 receptor activation potentiated DHPG-evoked intracellular calcium responses in cultured cortical type 2 astrocytes. In line with this, Ciruela and coworkers (2001) [32] by using co-transfected HEK-293 cells with the cDNAs for $mGlu_{1a}$ and A_1 receptors showed that the activation of $mGlu_{1a}$ potentiated the calcium peak obtained in response to A_1 activation. Similarly, they also found that A_1 receptor activation increased the calcium responses induced by $mGlu_1$ activation. Inhibitory interactions between the two groups of receptors have also been reported. Thus, the measurement of intracellular cAMP in several clones with inducible expression of A_1 and $mGlu_1$ receptors (HEK293am cells) revealed that $mGlu_1$ activation decreased A_1 receptor signalling measured as adenylate cyclase inhibition [33] whereas the activation of the A_1 receptor in Purkinje cells lead to the depression of $mGlu_1$-evoked calcium responses.

In vivo experiments also revealed the functional interaction between mGlu and adenosine receptors in rodent models. Thus, our research group showed that chronic subcutaneous injection of R-N^6- phenylisopropiladenosine (R-PIA), a selective A_1 receptor agonist, for 6 days in male rats evoked an increase in group I-mGlu receptors functionality which was also accompanied by an up-regulation of mGlu receptor number [34]. Chronic oral consumption of R-PIA during gestation also altered group I-mGlu receptor functionality in plasma membrane from the maternal brain. However, in this case, we observed a significant decrease of $mGlu_1$ and $mGlu_5$ receptors and a corresponding desensitization of mGlu receptor/PLC signalling pathway [35] which suggest that crosstalk processes may be influenced by different parameters such as gender or routes of administration. In reciprocal experiments, continuous L-glutamate intake throughout gestation also decreased A_1 receptor/AC signalling pathway in the maternal brain [36]. Finally, we have observed not only changes in group I-mGlu receptors functionality upon prolonged exposure to A_1 receptor agonist but also we showed a loss of group I-mGlu receptors responsiveness following chronic caffeine exposure which suggests that the blockade of adenosine receptors might also alter mGlu receptor functionality [37].

There are multiple works showing a negative interaction between A_1 and $mGlu_1$ receptors at the level of synaptic transmission. Thus, Tabata and coworkers (2007) showed that the activation of A_1 depressed $mGlu_1$ signalling measured as $mGlu_1$-coupled inward cation current and $mGlu_1$-coupled calcium release from intracellular stores in Purkinje cells [38]. Similarly, Kamikubo and coworkers (2013) also demonstrated that the activation of A_1 in cultured mouse Purkinje cells blocked $mGlu_1$-mediated long-term depression of glutamate-responsiveness [39]. Reciprocal negative interaction between mGlu and A_1 receptors has also been demonstrated in: (1) cerebrocortical synaptosomes where an agonist of group I/II mGlu receptors suppressed the inhibitory action of A_1 on glutamate release [40]; and (2) in rat hippocampal slices where electrophysiological recordings showed that the activation of $mGlu_{1a}$ suppressed A_1-mediated responses [41].

2.2. Adenosine A_2 and Metabotropic Glutamate Receptors Cross-talk

A positive interaction between A_{2A} and $mGlu_5$ receptors has been demonstrated in the ventral striatopallidal pathway, striatum and hippocampus where the activation of A_{2A} in the nucleus accumbens and in glutamatergic nerve terminals of the rat striatum facilitated GABA and L-glutamate release, respectively [27, 42]. On the other hand, in neostriatal slices, both receptors acted in a synergistic way by phosphorylating DARPP-32 [43]. Interestingly, there are no data reporting functional interaction at the level of the second messenger between A_{2A} and $mGlu_5$ receptors although complexes containing A_{2A} and $mGlu_5$ have been described in heterologous systems and in native tissues and functional interaction has also been revealed on ERK 1/2 phosphorylation, c-fos expression and neurotransmitter release [44]. The synergy between A_{2A} and $mGlu_5$ also affect other receptors such as NMDA receptor or dopamine D_2 receptor (D_2R). In the case of NMDA receptor, it was described how adenosine A_{2A} enhanced $mGlu_5$-mediated potentiation of NMDA effects in the striatum and hippocampus [45, 46]. Concerning D_2R, both receptors acted synergistically to reduce the affinity of D_2R and to modulate D_2R-mediated control of striatopallidal GABA neurons [47, 48].

Striatal region plays a fundamental role in motor and in reward, motivation and drug-seeking behaviour. As shown above, there are multiple works reporting a functional interaction between A_{2A} and $mGlu_5$ receptors in the striatum. It is reasonable that the interaction between the two groups of receptors at a behavioural level has been focused on animal models of Parkinson disease (PD) and in abuse drug-induced mechanisms. Thus, Coccurello and coworkers (2004) observed that perfusion of 6-hydroxydopamine in the striatum of rats produced akinetic deficits [49]. These deficits were not abolished when rats were treated with a non-effective dose of MPEP, a selective $mGlu_5$ antagonist. Similar results were found when hydroxydopamine rats were exposed to a non-effective dose of CSC, a selective A_{2A} antagonist. However, coadministration of both antagonists promoted a full recovery of akinesia demonstrating a behavioural interaction between A_{2A} and $mGlu_5$. Kachroo and coworkers (2005) also found that a simultaneous blockade of A_{2A} and $mGlu_5$ receptors acutely stimulates motor activity in a synergistic way in parkinsonian mice [50]. Interestingly, authors reinforced the importance of developing novel and efficient dual antagonist in order to avoid that the basal activity of $mGlu_5$ may hamper the antiparkinsonian potential of A_{2A} antagonist administered alone.

Concerning the role of A_{2A}-$mGlu_5$ interactions in drug-seeking behaviour, Brown and coworkers (2012) showed that this interaction participates in mediating the conditioned effects of cocaine but not cocaine-induced hyperactivity [51]. Thus, by using a combination of pharmacological and

genetic approaches, authors found that the selective $mGlu_5$ antagonist MTEP prevented cocaine-conditioned place preference in wild-type mice but failed in A_{2A} KO mice. The functional interactions between A_{2A} and $mGlu_5$ have also been shown to regulate alcohol-seeking in a rodent model of alcohol use. Thus, a combination of subthreshold doses of MTEP, $mGlu_5$ antagonist, and SCH 58261, A_{2A} antagonist, induced a higher reduction in alcohol-self administration compared to vehicle or either antagonist alone [52]. Finally, the interaction A_{2A}-$mGlu_5$ has also been shown to modulate the locomotor, stereotypic and drug-seeking effect of methamphetamine. In this case, authors also used a combination of subthreshold doses of MTEP and SCH 58261 and found that antagonist combination prevented methamphetamine-induced locomotor and stereotypic rearing responses and methamphetamine-seeking behaviors [53].

3. POSSIBLE MECHANISMS OF CROSSTALK BEHIND FUNCTIONAL INTERACTION

In general, the mechanisms through which adenosine and mGlu receptors interact are poorly investigated. We present here a range of mechanisms suggested to participate in some of the functional interactions described in the above paragraphs, including direct association of receptors (di/oligomerization), phosphorylation by second-messengers dependent protein kinases (*e.g*. protein kinase A or PKA, protein kinase C or PKC), and sharing of G proteins or other cytoskeletal and/or adaptor proteins.

A signalling pathway can be considered as a succession of interactions between proteins that result in the generation of a cellular response. Such interactions must occur in an orderly and coordinated way, otherwise the normal functioning of the cellular signalling would be lost, probably leading to diseases such as cancer or Alzheimer's. These interactions are part of the so-called *interactomics* of the cell. Protein–protein interactions are the physical contacts of high specificity established between two or more proteins as a result of biochemical events steered by electrostatic forces including the hydrophobic effect [54]. These interactions may last a short time or a long time, or be the basis of the formation of macromolecular complexes.

It is easy to imagine that thousands of interactions between different proteins can occur in the interior of a cell and that to generate a list of all of them can be a huge work. The combination of the available data about these interactions, not only between proteins but between the different components of a cell, generates such a large quantity of data that it becomes necessary (essential) to develop large databases and the appropriate tools to access meaningful data. In fact, availability and accessibility of these databases to the scientific community are increasing. Sometimes the information is redundant, that is, there is not a single database in which all

Table 1. List of consulted databases with corresponding webpage links (URL) and other available information such as number of interactions and version of database.

Name	Interactions	Version	URLs
APID Interactomes	1,225,163	1.1.5	http://apid.dep.usal.es/
BAR	115,325	1.3.13	http://bar.utoronto.ca/
BindingDB	1,011,029	v1.3	http://www.bindingdb.org
BioGrid	1,384,042	1.3.9	http://www.thebiogrid.org
ChEMBL	628,504	1.2.7	https://www.ebi.ac.uk/chembl/
DIP	104,965	1.3	http://dip.doe-mbi.ucla.edu/dip/Main.cgi
GPCR-HetNet	537	-	http://gpcr-hetnet.com/
HPIDb	4,763	1.3.13	http://hpidb.igbb.msstate.edu/main.html
I2D	817,915	1.1.6	http://ophid.utoronto.ca/ophidv2.204/
IntAct	589,538	1.3.13	https://www.ebi.ac.uk/intact/
iRefWeb	-	13.0	http://wodaklab.org/iRefWeb/search/index
MatrixDB	1,342	1.3.13	http://matrixdb.univ-lyon1.fr/
Mentha	1,144,545	-	http://mentha.uniroma2.it/
MINT	123,717	1.3.13	http://mint.bio.uniroma2.it/
Reactome-FIs	209,988	1.2.7	http://reactome.org/
STRING	1,380,838,440	10.5	https://string-db.org/
UniProt	14,968	1.3.13	http://www.uniprot.org/
ZINC	19,811	1.3.13	http://zinc15.docking.org/

Table 2. List of gene and protein (Uniprot ID) names of receptors reviewed here. PDB: Protein Data Bank code is indicated only for the available experimental (not predicted by in silico modelling) 3D structure.

	Homo sapiens			*Rattus norvegicus*		
Description	**Gene Name**	**Uniprot ID**	**PDB**	**Gene Name**	**Uniprot ID**	**PDB**
Adenosine receptor A_1	ADORA1	P30542	5N2S	Adora1	P25099	
Adenosine receptor A_{2A}	ADORA2A	P29274	3EML	Adora2a	P30543	
Adenosine receptor A_{2B}	ADORA2B	P29275		Adora2b	P29276	
Adenosine receptor A_3	ADORA3	P0DMS8		Adora3	P28647	
Metabotropic glutamate receptor 1	GRM1	Q13255	4OR2	Grm1	P23385	1EWK
Metabotropic glutamate receptor 5	GRM5	P41594	4OO9	Grm5	P31424	

these interactions are collected, but at least several of them can be combined to obtain the most relevant information available. In line with this, The Proteomics Standard Initiative Common QUery InterfaCe (PSICQUIC) specification was created by the Human Proteome Organization Proteomics Standards Initiative (HUPO-PSI) in 2008. Its web service provides computational access to molecular-interaction data resources. The PSICQUIC protein interaction datasets are accessible directly from the Cytoscape molecular interaction network tool, allowing users to select the interaction data from all or a subset of the datasets available. In the present review, we used Cytoscape 3.5.1 tool, an open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data [55]. Through its *Universal Interaction Database Client* searching tool, we collected data about interactions where any of the receptors reviewed here were involved. Other database sources of protein interactions were manually browsed. All of them have been summarized in Table **1**.

It must be noted that many of adenosine and mGlu receptor proteins reviewed here can be identified with more than one name (Table **2**). Therefore, searching started with the 'official' proposed name (UniProt ID) but its synonyms (see www.uniprot.org) were also employed to browse databases, including Worldwide Protein Data Bank (https://www.wwpdb.org) [56].

3.1. Direct Interaction between Adenosine and Metabotropic Glutamate Receptors

The protein-protein interactions have been studied by different methodological approaches and many of them can be recovered from the public online databases cited above. Fig. (**1**) shows a direct interactions network established between adenosine and group I metabotropic glutamate receptors in *Homo sapiens*, according to available scientific data, that could contribute to the *crosstalk* modulation reviewed herein (Table **3**). These direct interactions have also been reported in *Rattus norvegicus*; even a direct interaction between rat $mGlu_5$ and human A_{2A} has been detected, probably due to a conserved structure in the latter receptor with respect to their homologue in the rat.

GPCRs function can be the result of their individual (monomer state) and/or aggregated activation (oligomerized state). This oligomerization with themselves (*homomers*) or different receptors (*heteromers*) can modulate its pharmacology and functionality [5]. Different experimental techniques have been devised to study GPCR oligomerization, which was deeply reviewed elsewhere [57]. GPCR homomers, mainly dimers, seems to be a more generalized feature for GPCR than expected [58]. In fact, several adenosine and metabotropic glutamate receptors are now known to form homodimers [59, 60], Moreover, such dimerization processes could be a key determinant of their activation and function. It has been proposed that mGlu receptors must be in a homodimer state to be activated by glutamate, as reported when a full-length $mGlu_2$ receptor was purified, reconstituted, and stabilized in a native-like environment [61]. This preferential formation of mGlu receptors homodimers at the surface of cells has been also reported by using a time-resolved Förster resonance energy transfer (trFRET) combined with SNAP-tag technology [62, 63] and by photobleaching experiments using GFP labeled mGlu receptors [64]. Higher order structures (*e.g*. dimers of dimers) seems to be very transient, as they have only been detected in the case of $mGlu_2$ when their interaction was stabilized by a disulfide bridge [65].

In turn, the formation of heteromers probably represents the easiest way through which different GPCRs can interact with them. Heterologous expression studies revealed that group I mGlu receptors on one hand, and group II and III mGlu receptors, on the other hand, could form heterodimers [63], increasing to 16 the number of additional mGlu receptors with likely specific pharmacological and functional properties.

The combination of co-immunoprecipitation, Western-blotting and immunocytochemical assays revealed that $mGlu_{1a}$ and A_1 assembled into functionally interacting complexes in rat cerebellum and in HEK-293 cells [32]. Similarly, co-immunoprecipitation, immunofluorescence and FRET assays also showed that A_1 and $mGlu_1$ receptors formed a complex in HEK293am cells [33]. The formation of this complex has been suggested to participate in the *crosstalk* process in which the activation of $mGlu_1$ inhibited A_1 functionality, measured as inhibition of forskolin-stimulated

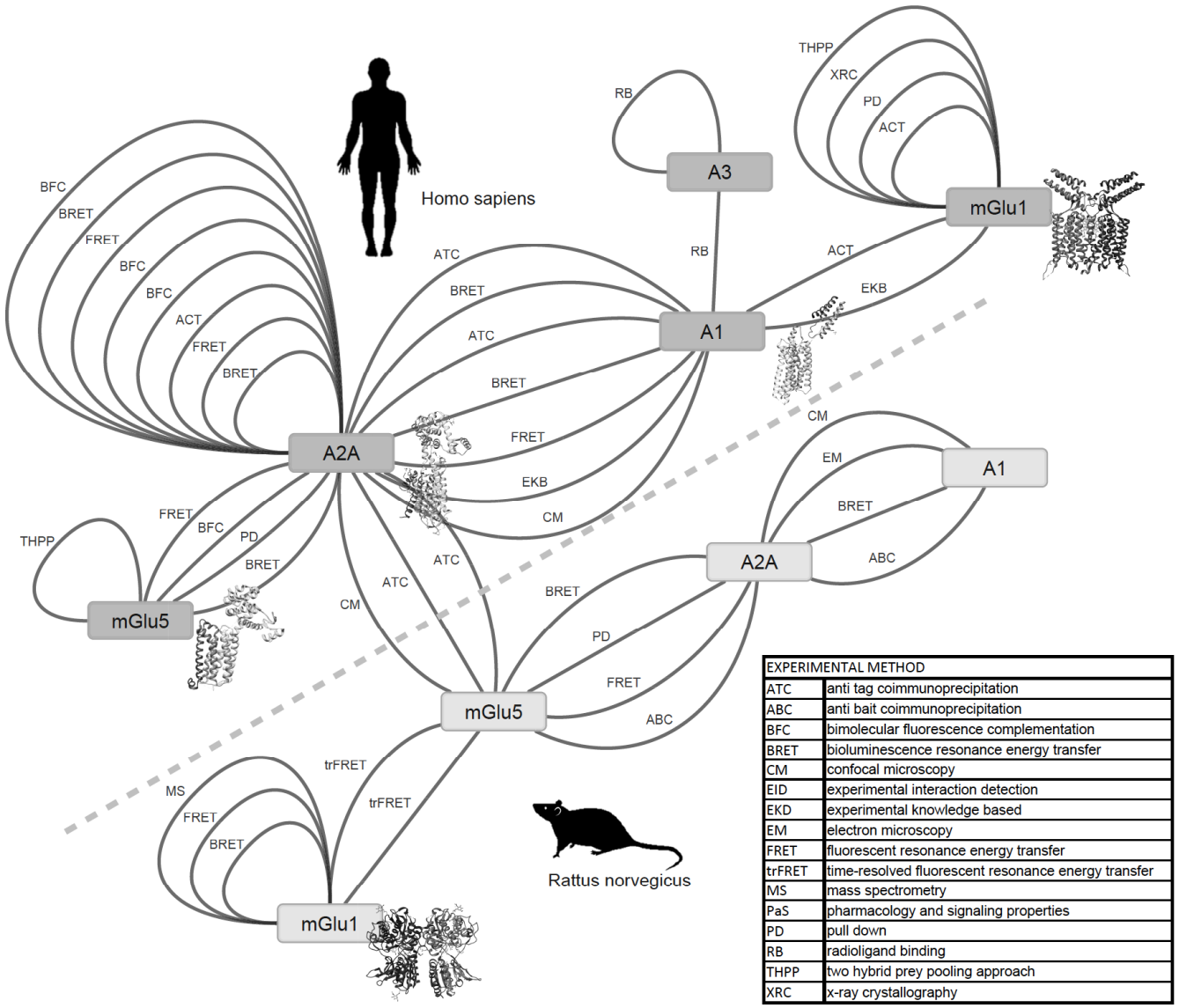

Fig. (1). Direct interaction network of adenosine receptors and group I metabotropic glutamate receptors. in *Homo sapiens* and *Rattus norvegicus*. Connecting lines between receptors (boxes) indicate the methodology (see insert) employed to assess such direct interaction as summarized in Table **3**. Green box: rat protein. Blue box: human protein. 3D structures were downloaded from Worldwide Protein Data Bank (https://www.wwpdb.org) [56] and they are summarized in Table **2**.

cAMP accumulation [33]. A_1 heteromerize with $mGluR_{1a}$, resulting in the modulation of glutamate cytotoxicity, while A_{2A} functionally interacts with $mGlu_5$ in the striatum, as supported by studies in cotransfected cells and knockout mice [9, 53, 66] but also in native rat striatum [22, 27].

3.2. Functional Cross-talk through the G-proteins

Guanine nucleotide-binding proteins (G proteins) are involved as modulators or transducers in various transmembrane signalling systems. Gα-subunits are divided into four families: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$. The $G\alpha_s$ and $G\alpha_{i/o}$ families activate or inhibit the activity of adenylate cyclase, respectively. The $G\alpha_{q/11}$ family stimulates phospholipase C (PLC). Finally, the $G\alpha_{12/13}$ family activates small GTPases including the Rho family (*e.g.*, RhoA) [67]. Thus, $mGlu_2$ and $mGlu_4$, two receptors negatively coupled to adenylyl cyclase, its canonical pathway, are able to activate PLC, its non-canonical pathway, when coexpressed with the G proteins $G\alpha_{15}$ or $G\alpha_q$ [68]. In ON bipolar neurons from retina $G\alpha_{o1}$ mediates $mGlu_6$ signalling [69]. Adenosine A_1 receptor also needs $G\alpha_{o1}$ binding to form the ternary complex between agonist, receptor, and G protein [70]. Another mechanism proposed that involves G protein was described in retinotectal transmission where both A_1 receptor and group II mGlu receptors mediated presynaptic inhibition at the retinotectal synapse of goldfish by sharing a $G\alpha_i$ protein. For that reason, when both groups of receptors were simultaneously activated the inhibitory effects on synaptic transmission were not additive but occluded each other [29]. The interaction with and the activation of these G proteins by GPCRs is a regulated process. Thus, a regulator of G-protein signalling 12 (RGS12)

Table 3. Experimentally determined protein-protein interactions in both *Homo sapiens* and *Rattus norvegicus* models. $mGlu_N$: metabotropic glutamate receptor N. A_N: adenosine A_N receptor. Empty cells: no data available.

	Protein A	Protein B	Method	Interaction Type	Source	Publication
Homo sapiens	A_1	A_{2A}	anti tag coimmunoprecipitation	within the same physical complex	IntAct (EBI-2904802)	Ciruela *et al*., 2006 (PMID:16481441)
			anti tag coimmunoprecipitation	within the same physical complex	IntAct (EBI-2904883)	
			bioluminescence resonance energy transfer	physical association	IntAct (EBI-2904906)	
			confocal microscopy	colocalization	IntAct (EBI-2904959)	
			fluorescent resonance energy transfer	oligomerization	GPCR-HetNet	Schicker *et al*., 2009 (PMID:18973777)
			bioluminescence resonance energy transfer	oligomerization, in transfected HEK-293 cells	GPCR-HetNet	Cristóvão-Ferreira *et al*., 2013 (PMID:23657626)
			experimental knowledge based			Wu *et al*., 2010 (PMID:20482850)
		A_3	radioligand binding	oligomerization	GPCR-HetNet	Hill *et al*., 2014 (PMID:24024783)
		$mGlu_1$	experimental knowledge based			Wu *et al*., 2010 (PMID:20482850)
			affinity chromatography technology	physical association	BioGRID (302761)	Ciruela *et al*., 2001 (PMID:11278325)
	A_{2A}	A_{2A}	fluorescent resonance energy transfer	physical association	IntAct (EBI-8491775)	Vidi *et al*., 2008 (PMID:19013155)
			bimolecular fluorescence complementation	physical association	IntAct (EBI-8491974)	
			bimolecular fluorescence complementation	physical association	IntAct (EBI-8492130)	
			fluorescent resonance energy transfer	physical association	BioGRID (258280)	Kamiya *et al*., 2003 (PMID:12804599)
			bioluminescence resonance energy transfer	physical association	IntAct (EBI-8041827)	Gandia *et al*., 2008 (PMID:18675812)
			bioluminescence resonance energy transfer	physical association	IntAct (EBI-8041845)	
			bimolecular fluorescence complementation	physical association	IntAct (EBI-8041898)	
			affinity chromatography technology	physical association	BioGRID (616424)	Burgueño *et al*., 2003 (PMID:12837758)
		$mGlu_5$	fluorescent resonance energy transfer	oligomerization, in transfected HEK-293 cells	GPCR-HetNet	Cabello *et al*., 2009 (PMID:19344374)
			bimolecular fluorescence complementation			
			bioluminescence resonance energy transfer			
			pull down			
	A_3	A_3	radioligand binding	allosteric interaction	PubMed	May *et al*., 2011 (PMID:21715680)
	$mGlu_1$	$mGlu_1$	affinity chromatography technology	physical association	BioGRID (316750)	Robbins *et al*., 1999 (PMID:10349865)
			pull down	physical association	BioGRID (280937)	Ray *et al*., 2000 (PMID:10945991)
			x-ray crystallography	physical association	IntAct(EBI-9636453)	Wu *et al*., 2014 (PMID:24603153)
			two hybrid prey pooling approach	molecular interaction	HPRD (Q13255)	Kitano *et al*., 2002 (PMID:11850456)

(Table 3) contd….

	Protein A	Protein B	Method	Interaction Type	Source	Publication
	$mGlu_5$	$mGlu_5$	two hybrid prey pooling approach	molecular interaction	HPRD (P41594)	Kitano *et al*., 2002 (PMID:11850456)
Rattus norvegicus	A_1	A_{2A}	electron microscopy	colocalization	IntAct (EBI-2905139)	Ciruela *et al*., 2006 (PMID:16481441)
			confocal microscopy	colocalization	IntAct (EBI-2905272)	
			anti bait coimmunoprecipitation	within the same physical complex	IntAct (EBI-2905309)	
			bioluminescence resonance energy transfer	physical association, in primary cultures of astrocytes	GPCR-HetNet	Cristóvão-Ferreira *et al*., 2013 (PMID:23657626)
	A_{2A}	$mGlu_5$	anti bait coimmunoprecipitation	within the same physical complex, in rat striatum	IntAct (EBI-2902881)	Ferré *et al*., 2002 (PMID:12189203)
			fluorescent resonance energy transfer	oligomerization, in rat striatum	GPCR-HetNet	Cabello *et al*., 2009 (PMID:19344374)
			bimolecular fluorescence complementation			
			bioluminescence resonance energy transfer			
			pull down			
	$mGlu_1$	$mGlu_1$	mass spectrometry study of hydrogen/deuterium exchange	molecular interaction	Bind (91134)	Kunishima *et al*., 2000 (PMID:11069170)
			fluorescent resonance energy transfer	physical association	IntAct (EBI-7090108)	Rives *et al*., 2009 (PMID:19590495)
			bioluminescence resonance energy transfer	physical association	IntAct (EBI-7090497)	
		$mGlu_5$	time-resolved fluorescence resonance energy transfer	physical association	PubMed	Goudet *et al*., 2005 (PMID:15863499)
			time-resolved fluorescence resonance energy transfer	physical association	PubMed	Doumazane *et al*., 2011 (PMID:20826542)
	$mGlu_5$ (rat)	A_{2A} (human)	anti tag coimmunoprecipitation	within the same physical complex, in transfected HEK-293 cells	IntAct (EBI-2902799)	Ferré *et al*., 2002 (PMID:12189203)
			anti tag coimmunoprecipitation	within the same physical complex, in transfected HEK-293 cells	IntAct (EBI-2902807)	
			confocal microscopy	colocalization, in transfected HEK-293 cells	IntAct (EBI-2902730)	

Source: identification code for the specified interaction at the indicated database (Table **1**). PMID: PubMed ID for the cited reference.

inhibits signal transduction by increasing the GTPase activity of Gα subunits, thereby driving them into their inactive GDP-bound form. RGS12 interacts with $G\alpha_{i1}$ [71], and surface plasmon resonance analysis of RGS12 PDZ domain binding specificity has revealed its interaction with $mGlu_1$, $mGlu_2$ and $mGlu_5$ receptors [72].

Not only can Gα-subunits mediate *crosstalk* processes between GPCRs. G protein βγ subunits have been also proposed as mediators of the enhanced group I mGlu-evoked $[Ca^{2+}]$ responses observed in type 2 astrocytes when adenosine A_1 receptors are co-activated [31], suggesting that phospholipase C activity could be increased by G protein βγ subunits although the precise mechanism was not investigated. However, other reported *crosstalk* processes seem to be G protein-independent. Thus, Tabata and coworkers (2007) by using a perforated-patch voltage-clamp technique in cerebellar Purkinje cells showed that A_1 activation induced a continuous depression of $mGlu_1$ [38]. This interaction did not require $G\alpha_{i/o}$ proteins. Finally, it has also been reported that $mGlu_5$ potentiated A_2 receptor signalling in neostriatal slices in a process in which mitogen-activated protein kinase (ERK) pathway seems to participate but not a synergism at the second messenger level [43, 44].

3.3. Functional Cross-talk through other Intracellular Proteins

The interaction of several GPCRs with specific proteins ('accessory proteins') can completely change their expression, function and fate. GPCRs themselves can also be accessory proteins when considering their homo- or heterodimers. Some of the main interacting proteins of GPCRs were reviewed elsewhere [73]. Adenosine and metabotropic glutamate receptors are also examples of GPCRs modulated by these interactions, and Table **4** summarizes several proposed linkers between the different receptors reviewed here. These linkers have been grouped according to their possible roles in receptor's crosstalk: cell surface expression, cytoskeletal linkage, G-protein coupling, GPCR regulation and GPCR degradation.

Table 4. Possible protein-linkers mediating adenosine and metabotropic glutamate receptors *crosstalk* at different levels, according to their possible roles in relation to GPCR function.

Role	Possible Linker	Linked Proteins	Authors	PubMed ID
Cell surface expression	ADA	A_1	Torvinen *et al*. (2002)	12150791
			Franco *et al*. (1997)	9247966
		A_{2A}	Herrera *et al*. (2001)	11125033
		A_{2B}		
	DRiP78	A_1	Málaga-Diéguez *et al*. (2010)	20219842
	Homer 1	$mGlu_1$	Ango *et al*. (2001)	11418862
			Brakeman *et al*. (1997)	9069287
Cytoeskeletal adapters		$mGlu_5$	Ango *et al*. (2001)	11418862
			Brakeman *et al*. (1997)	9069287
	Cyth2	A_{2A}	Gsandtner *et al*. (2005)	16027149
		$mGlu_1$	Kitano *et al*. (2002)	11850456
	Efnb2	$mGlu_1$	Caló *et al*. (2005)	15745950
		$mGlu_5$		
	FLNA	$mGlu_{5a}$	Enz *et al*. (2002)	11943148
		$mGlu_{5b}$		
	Necab2	A_{2A}	Canela *et al*. (2009)	19694902
			Canela *et al*. (2007)	17689978
		$mGlu_5$	Canela *et al*. (2009)	19694902
	NHERF1-2	$mGlu_5$	Paquet *et al*. (2006)	16891310
		A_{2B}	Sitaraman *et al*. (2002)	12080047
G protein coupling	$G\alpha_{o1}$	A_1	Waldhoer *et al*. (1999)	10521440
		$mGlu_6$	Dhingra *et al*. (2000)	11124982
	RGS12	$mGlu_1$	Snow *et al*. (1998)	9651375
		$mGlu_5$		
	$G_{\beta\gamma}$	A_1	Toms and Roberts, (1999)	10530813
		$mGlu_1$		
		$mGlu_5$		
GPCR Regulation	CaM	A_{2A}	Navarro *et al*. (2009)	19632986
		$mGlu_5$	Ko *et al*. (2012)	23152621
			Saugstad *et al*. (2002)	11953448
			Minakami *et al*. (1997)	9242710
	GRASP	$mGlu_1$	Kitano *et al*. (2002)	11850456
		$mGlu_5$		

Table 4. contd….

Role	Possible Linker	Linked Proteins	Authors	PubMed ID
GPCR Regulation	Pick1	$mGlu_1$	Gan *et al*. (2016)	26946972
		$mGlu_5$		
	PKA	A_{2A}	Tebano *et al*. (2006)	18404464
		$mGlu_5$		
	PKC	$mGlu_1$	Francesconi *et al*. (2000)	10823959
		$mGlu_5$	Ko *et al*. (2012)	23152621
			Minakami *et al*. (1997)	9242710
		A_2	Cartmell *et al*. (1998)	9593890
		$mGlu_1$		
		$mGlu_5$		
		A_1	de Mendonça *et al*. (1997)	9283686
		$mGlu_1$		
		$mGlu_5$		
		A_1	Shahraki *et al*. (2003)	12684261
		$mGlu_1$		
GPCR Degradation	Gasp-1	$mGlu_1$	Heydorn *et al*. (2004)	15452121
		$mGlu_5$		
	Ubiquitin C	A_{2B}	Thompson *et al*. (2014)	25147182
		$mGlu_5$	Wagner *et al*. (2011)	21890473

Adenosine deaminase (ADA) enzyme acts as a positive modulator of adenosine A_1 and A_{2A} receptors, by enhancing their ligand affinity *via* conformational change [74]. Interestingly, ADA can interact with, and mediate the interaction between, A_1 [75, 76], A_{2A} and A_{2B} receptors at the cell surface [77]. Moreover, binding of ADA to A_{2B} receptors increases the affinity of the agonist 5'-N-ethylcarboxamidoadenosine and cAMP production [77]. In turn, the ER-associated 40-kDa heat shock protein family member D_1 receptor interacting protein 78 (DRiP78) physically interacts and retain adenosine A_1 receptor in ER, and negatively regulates the cell-surface expression of this adenosine receptor [78] avoiding its interaction with other GPCRs at the cell surface.

Homer protein homolog 1 (Homer 1) is a postsynaptic density scaffolding protein which binds and cross-links cytoplasmic regions of $mGlu_1$ and $mGlu_5$ [79, 80] and regulates the trafficking and surface expression of these receptors. By physically linking $mGlu_1$ and $mGlu_5$ with ER-associated ITPR1 receptors, Homer 1 aids the coupling of surface receptors to intracellular calcium release [81].

NHERF1-2 can bind $G\alpha_q$-subunit, PLC and PKC, suggesting that NHERFs act as a scaffold protein bringing the effectors of the pathway together to facilitate the signal transmission. NHERF2 has also been shown to bind to the

$mGlu_5$ [82], where NHERF2 also potentiates the receptor-induced PLC activation, prolonging the intracellular Ca^{2+} mobilization. Moreover, NHERF1 and NHERF2 contribute to regulating the distribution of $mGlu_2$ and $mGlu_3$ in the murine brain [83].

Filamin-A (FLNA) promotes orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins. Anchors various transmembrane proteins to the actin cytoskeleton and serves as a scaffold for a wide range of cytoplasmic signalling proteins. Some of these proteins are $mGlu_{4a}$, $mGlu_{5a}$, $mGlu_{5b}$, $mGlu_{7a}$, $mGlu_{7b}$ and $mGlu_{8a}$, where a conserved tyrosine within mGlu receptor carboxi termini mediates the binding to FLNA [84].

N-terminal EF-hand calcium-binding protein 2 (Necab2) may act as a signalling scaffold protein that senses intracellular calcium. The interaction of Necab2 with adenosine A_{2A} receptor modulates the cell surface expression, the ligand-dependent internalization and the receptor-mediated activation of the MAPK pathway [85]. Moreover, Necab2 modulates receptor function and coupling efficiency of $mGlu_5$ by its physical interaction with this receptor [86].

Crosstalk between adenosine and metabotropic glutamate receptors can involve second-messengers dependent protein kinases. Thus, it is well known that in Central Nervous System, A_{2A} is coupled to G_s proteins, and stimulates the activity of adenylate cyclase which increases cyclic AMP production leading consequently to an increase in protein kinase A activity (PKA). It has also been described how PKA, by phosphorylating the aminoacid residue Ser870 located within the calmodulin binding site I in $mGlu_5$, regulated the function of $mGlu_5$ assayed as such as extracellular signal-regulated kinase phosphorylation and intracellular Ca^{2+} oscillations [87]. Finally, the participation of PKA in the crosstalk process between A_{2A} and $mGlu_5$ has been shown in striatal slices where the facilitatory role of A_{2A} receptors on $mGlu_5$-mediated responses was abolished by PKA inhibitor [46]. Interestingly, the same work also revealed that the participation of PKA seemed to be brain region-dependent since the synergism between the two receptors in hippocampal slices was resistant to PKA inhibitor [46].

Concerning group I mGluR, both receptor subtypes $mGlu_1$ and $mGlu_5$ are coupled in a positive way to phospholipase C through G_q proteins leading to triggering of inositol-1,4,5-trisphosphate (IP_3)-mediated calcium release and to activation of protein kinase C (PKC). The ability of PKC to phosphorylate A_{2A} receptor has been shown in rat C6 glioma cells where the phosphorylation of receptors on three different aminoacid residues (Thr298, Ser320, and Ser335) was increased by pretreatment with PKC activator although no variations in A_{2A} receptor signalling were detected [88]. However, PKC has been suggested as participating in the synergism observed between group I mGlu and adenosine receptors in primary cultures of rat striatal neurons. Thus, in these cells, the potentiation of adenosine receptor responses observed upon group I mGlu receptors activation was abolished by a PKC inhibitor [89]. On the other hand, several works using rat hippocampal slices found that $mGlu_{1a}$ receptor abolished A_1 receptor-mediated responses in a process in which PKC seemed to be involved [41, 90, 91].

The functional effects cited above could be mediated by physical interactions with proteins such as calmodulin, GRP1 or Pick1, which together with PKC and PKA would contribute to GPCR regulation. Calmodulin (CaM) mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins through calcium-binding. The carboxi terminal domain of $mGlu_5$ and $mGlu_7$ has been shown to bind to CaM [92, 93] and immobilized-CaM beads pulled down $mGlu_3$, $mGlu_{4a}$ and $mGlu_7$ immunoreactivity from solubilized rat brain membranes in a Ca^{2+}-dependent manner [94]. On $mGlu_7$ only one CaM-binding site exists, whereas $mGlu_5$ binds CaM at two distinct sites [95]. Moreover, CaM competes with the E3 ligase seven in absentia homolog (Siah)-1A for $mGlu_5$ binding in a phosphorylation-dependent manner in rat hippocampal neurons, dynamically regulating $mGlu_5$ trafficking [96]. In turn, CaM-binding to $mGlu_7$ [93] overlaps the PKC phosphorylation site; thus calcium/CaM binding to the receptor inhibits PKC phosphorylation of $mGlu_7$ [97-99]. It has been reported that CaM binds preferentially to a proximal C-terminus epitope of the adenosine A_{2A} receptor [100]. Thus, Ca^{2+} seems to induce conformational changes in the CaM-A_{2A}-D_2 receptor oligomer which leads to a selective modulation of MAPK signalling mediated by the A_{2A}/D_2 receptor heteromer [100]. A similar interaction could occur between A_{2A} and other mGlu receptors such as $mGlu_5$.

General receptor for phosphoinositides 1-associated scaffold protein (GRP1-associated scaffold protein or GRASP) interacts with group I metabotropic glutamate receptors ($mGlu_1$ and $mGlu_5$). The yeast two-hybrid system and *in vitro* pull-down assays indicated that the PDZ (PSD-95/Dlg1/ZO-1) domain-containing, amino-terminal half of GRASP directly binds to the class I PDZ-binding motif of group I mGlu receptors [101]. The C-terminal half of GRASP also bound to cytohesins, the members of guanine nucleotide exchange factors (GEFs) specific for the ADP-ribosylation factor (ARF) family of small GTP-binding proteins. In turn, cytohesin-2 (Cyth2) interacts with the adenosine A_{2A} receptor [102] leading to the activation of mitogen-activated protein (MAP) kinase, *via* a pathway that is independent of heterotrimeric G proteins. Native $mGlu_{1a}$/GRASP/cytohesin-2 complexes have also been coimmunoprecipitated from rat brain tissue [101].

$MGlu_{1a}$ receptor signalling *via* $InsP_3$ production induces the activation of PKC which, in turn, phosphorylates a threonine residue involved in receptor coupling to $G_{q/11}$, resulting in the desensitization of $mGlu_{1a}$ [103]. PKC is also involved in phosphorylation-dependent trafficking of $mGlu_5$ [92, 96] and the phosphorylation of $mGlu_7$ [104]. PKCα type (PKC-A) is a classical (or conventional) PKC activated by calcium and diacylglycerol in the presence of phosphatidylserine, and interacts with protein interacting with C-kinase (Pick1) *via* PDZ domain [105]. In addition, Pick1, a synaptic organizer protein, binds to the C terminus of $mGlu_{7a}$ [104, 106] and $mGlu_3$ [106]. Pick1 probably acts as an adapter protein that binds to and organizes the subcellular localization of a variety of membrane proteins containing some PDZ recognition sequence. In the agreement, Pick1 modulates PKCα-evoked phosphorylation of $mGlu_{7a}$ and is involved in

the clustering of various receptors, including $mGlu_7$, at presynaptic release sites [107], possibly by acting at the receptor internalization level [104].

Apart from interactions with proteins that modulate GPCR cell surface expression, G-protein coupling, cell localization and regulation, other partners could mediate its trafficking and final degradation. Many GPCRs require post-translational modification with ubiquitin and interaction with ubiquitin-binding domains of the endosomal-sorting complex required for transport (ESCRT) machinery for lysosomal sorting. In line with this, ubiquitin-C exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins *via* an isopeptide bond either as a monomer (monoubiquitin), a polymer linked *via* different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked *via* the initiator Met of the ubiquitin (linear polyubiquitin chains). This binding of ubiquitin to an Lys residue determines different protein consequences on function (*e.g*. protein degradation). Quantitative Lys-ϵ-Gly-Gly (diGly) proteomics aimed to identify ubiquitin ligases substrates revealed the interaction of adenosine A_{2B} receptor with ubiquitin [108]. Similar proteomic approaches also revealed the interaction of ubiquitin with different plasma membrane receptors, including $mGlu_5$ [109] and $mGlu_7$ [110]. However, not all GPCRs require direct ubiquitination or all components of the ESCRT machinery for degradation in the lysosome, suggesting that alternate sorting pathways exist [111]. One of them is GPCR lysosomal sorting that is regulated by the G protein-coupled receptor associated sorting protein-1 (Gasp-1), which modulates lysosomal sorting and functional down-regulation of a variety of GPCRs. Gasp-1 targets receptors for degradation in lysosomes *via* its interaction with BECN2 [112] and interacts with cytoplasmic tails of a variety of GPCRs such as D_2 and D_4 dopamine receptors, delta opioid receptor 1 and beta-2 adrenergic receptor. Interestingly, Gasp-1 also interacts with carboxi terminal domains from $mGlu_{1a}$, $mGlu_{5a}$, and $mGlu_8$ [113], probably contributing to the fate of these receptors in the cell.

Taken together, these functional and physical interactions with proteins cited above comprise the participation of cytoskeletal proteins and/or adaptor proteins that modify receptor localization, activation, trafficking and regulation, and participate in the *crosstalk* between these receptors.

4. NEW DRUG DESIGN POSSIBILITIES: TARGETING GPCR OLIGOMERS

According to previous paragraphs, GPCRs function is modulated through oligomer formation and protein-protein interactions. Therefore, a deeper knowledge about all these molecular interactions would be very helpful for the design of new, selective and therapeutically potent active drugs targeting these receptors, especially in the field of neurodegenerative disease [6]. Fine tuning of GPCR function through protein-protein interactions (*e.g*. homomerization or heteromerization) would comprise ligand interactions, cellular trafficking, and mobility [114, 115].

4.1. GPCR Homomerization can Modulate or Create new Binding Sites

Besides the orthosteric site in a monomeric GPCR, the assembly of two monomers could result in new binding sites in the dimeric form of the receptor. In addition, when a ligand binds to the second protomer in a homodimer, it will often act as an allosteric modulator of the intrinsic efficacy of the ligand when binding to the first protomer [115]. Accordingly, it has been reported that adenosine A_1 receptors form homomers not only in cell cultures but also in bovine brain cortex and that caffeine binding to one protomer increases the agonist affinity for the other protomer, a pharmacological characteristic that correlates with the low caffeine concentrations-induced activation of agonist-promoted A_1 receptor signalling [116].

4.2. GPCR Heteromerization-mediated Transantagonism and Transactivation

In the heteromeric form, the activation (or blockade) of a GPCR could modulate the function of its partner. For instance, the stimulation of adenosine A_{2A} receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes, where they heteromerize. This A_{2A}/D_2 heteromer may underlie the neuroleptic-like actions of adenosine agonists and the enhancing effects of adenosine antagonists on locomotor activity [117]. This heteromer represents the new concept of '*transantagonism'* when an oligomer interaction can occur in such a way that the activation of one receptor inhibits the signalling activity of the other. Another mechanism of *crosstalk* between GPCR heteromers would be '*transactivation'*, which occurs when the signalling cascade of one receptor is initiated upon an agonist binding to the other protomer [115]. These oligomers have been discussed as potential therapeutic targets for schizophrenia and/or Parkinson's disease as more specific alternatives to existing pharmaceuticals that target only the D_2 monomer [118]. $A_{2A}/mGlu_5$ heteromer function also depends on the activation of one or both protomers. Thus, the stimulation of the $mGlu_5$ receptor potentiates A_{2A} signalling in a MAPK-dependent manner in striatal slices [43] and could be mediated by the formation of $A_{2A}/mGlu_5/D_2$ receptor heteromers [119]. An interesting possibility is the simultaneous activation of two different GPCRs, when heteromerized, through bivalent ligands, as has previously been evaluated in the case of A_1/D_1 [120], A_{2A}/D_2 [121] or μ opioid receptor/$mGlu_5$ [122] heteromers.

4.3. GPCR Oligomerization-modulated G-protein Coupling

Finally, a potential result of GPCR oligomerization is the activation of different pathways from that activated by the monomeric state, for instance by novel G-protein coupling [123]. In line with this, the primary coupling of the group I mGluRs is with $G\alpha_{q/11}$; however, these receptors can couple with $G\alpha_s$ and $G\alpha_{i/o}$ as well when overexpressed, probably forming receptor mosaics, in HEK293 and CHO cells [124]. Although the binding of two G proteins to a heterodimer seems not feasible due to steric clashes [125], the A_1-A_{2A} heterotetramer (one A_1 homodimer plus one A_{2A} homodimer)

may bind to both $G\alpha_s$ and $G\alpha_i$ proteins [126], suggesting receptor oligomerization dynamics as a crucial point in the determination of G-protein coupling and activation.

CONCLUSION

From the previous paragraphs, we may draw some conclusions and lines of future research. Related to the envisaged work to do in the GPCR field, it seems mandatory: i) to develop new tools for the analysis of protein-protein interactions in the context of cell signalling, and ii) to improve the availability and accessibility of meaningful data through curated non-overlapping databases. To this end, it would be desirable to reduce the multiplicity of names used for the same protein/gene or for the same kind of data, and also to promote global initiatives similar to the above mentioned PSICQUIC. Finally, it is clear that signalling possibilities of a GPCR are not only limited to classical and linear signalling pathway activation (or blockade) but are impressively increased and diversified as a result of the variety of partners with which that GPCR could interact. The immediate consequence is the higher complexity that research on GPCR signalling deserves. But paradoxically, this difficulty should envisage an optimistic future as there would also be newer possibilities to target this GPCR in pathological status and disease.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We thank Tom Yohannan for editorial assistance.

REFERENCES

[1] Cattaneo, F.; Guerra, G.; Parisi, M.; De Marinis, M.; Tafuri, D.; Cinelli, M.; Ammendola, R. Cell-surface receptors transactivation mediated by g protein-coupled receptors. *Int. J. Mol. Sci.,* **2014**, *15*(11), 19700-19728. [http://dx.doi.org/10.3390/ijms151119700] [PMID: 25356505]

[2] Rivero-Müller, A.; Jonas, K.C.; Hanyaloglu, A.C.; Huhtaniemi, I. Di/oligomerization of GPCRs-mechanisms and functional significance. *Prog. Mol. Biol. Transl. Sci.,* **2013**, *117*, 163-185. [http://dx.doi.org/10.1016/B978-0-12-386931-9.00007-6] [PMID: 23663969]

[3] Rajagopal, S.; Shenoy, S.K. GPCR desensitization: Acute and prolonged phases. *Cell. Signal.,* **2018**, *41*, 9-16. [PMID: 28137506]

[4] Borroto-Escuela, D.O.; Brito, I.; Romero-Fernandez, W.; Di Palma, M.; Oflijan, J.; Skieterska, K.; Duchou, J.; Van Craenenbroeck, K.; Suárez-Boomgaard, D.; Rivera, A.; Guidolin, D.; Agnati, L.F.; Fuxe, K. The G protein-coupled receptor heterodimer network (GPCR-HetNet) and its hub components. *Int. J. Mol. Sci.,* **2014**, *15*(5), 8570-8590. [http://dx.doi.org/10.3390/ijms15058570] [PMID: 24830558]

[5] Farran, B. An update on the physiological and therapeutic relevance of GPCR oligomers. *Pharmacol. Res.,* **2017**, *117*, 303-327. [http://dx.doi.org/10.1016/j.phrs.2017.01.008] [PMID: 28087443]

[6] Dalet, F.G.; Guadalupe, T.F.; María Del Carmen, C.H.; Humberto, G.A.; Antonio, S.U. Insights into the structural biology of G-protein coupled receptors impacts drug design for central nervous system neurodegenerative processes. *Neural Regen. Res.,* **2013**, *8*(24), 2290-2302. [PMID: 25206539]

[7] Burford, N.T.; Wehrman, T.; Bassoni, D.; O'Connell, J.; Banks, M.; Zhang, L.; Alt, A. Identification of selective agonists and positive allosteric modulators for μ- and δ-opioid receptors from a single high-throughput screen. *J. Biomol. Screen.,* **2014**, *19*(9), 1255-1265. [http://dx.doi.org/10.1177/1087057114542975] [PMID: 25047277]

[8] Chen, J.F.; Eltzschig, H.K.; Fredholm, B.B. Adenosine receptors as drug targets--what are the challenges? *Nat. Rev. Drug Discov.,* **2013**, *12*(4), 265-286. [http://dx.doi.org/10.1038/nrd3955] [PMID: 23535933]

[9] Ciruela, F.; Gómez-Soler, M.; Guidolin, D.; Borroto-Escuela, D.O.; Agnati, L.F.; Fuxe, K.; Fernández-Dueñas, V. Adenosine receptor containing oligomers: their role in the control of dopamine and glutamate neurotransmission in the brain. *Biochim. Biophys. Acta,* **2011**, *1808*(5), 1245-1255. [http://dx.doi.org/10.1016/j.bbamem.2011.02.007] [PMID: 21316336]

[10] Nakata, H.; Suzuki, T.; Namba, K.; Oyanagi, K. Dimerization of G protein-coupled purinergic receptors: increasing the diversity of purinergic receptor signal responses and receptor functions. *J. Recept. Signal Transduct. Res.,* **2010**, *30*(5), 337-346. [http://dx.doi.org/10.3109/10799893.2010.509729] [PMID: 20843271]

[11] Fuxe, K.; Borroto-Escuela, D.O.; Marcellino, D.; Romero-Fernandez, W.; Frankowska, M.; Guidolin, D.; Filip, M.; Ferraro, L.; Woods, A.S.; Tarakanov, A.; Ciruela, F.; Agnati, L.F.; Tanganelli, S. GPCR heteromers and their allosteric receptor-receptor interactions. *Curr. Med. Chem.,* **2012**, *19*(3), 356-363. [http://dx.doi.org/10.2174/092986712803414259] [PMID: 22335512]

[12] Franco, R.; Casadó, V.; Cortés, A.; Ferrada, C.; Mallol, J.; Woods, A.; Lluis, C.; Canela, E.I.; Ferré, S. Basic concepts in G-protein-coupled receptor homo- and heterodimerization. *Sci. World J.,* **2007**, *7*, 48-57. [http://dx.doi.org/10.1100/tsw.2007.197] [PMID: 17982576]

[13] Zezula, J.; Freissmuth, M. The A(2A)-adenosine receptor: a GPCR with unique features? *Br. J. Pharmacol.,* **2008**, *153*(Suppl. 1), S184-S190. [http://dx.doi.org/10.1038/sj.bjp.0707674] [PMID: 18246094]

[14] Julio-Pieper, M.; Flor, P.J.; Dinan, T.G.; Cryan, J.F. Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. *Pharmacol. Rev.,* **2011**, *63*(1), 35-58. [http://dx.doi.org/10.1124/pr.110.004036] [PMID: 21228260]

[15] Pin, J.P.; Acher, F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr. Drug Targets CNS Neurol. Disord.,* **2002**, *1*(3), 297-317. [http://dx.doi.org/10.2174/1568007023339328] [PMID: 12769621]

[16] Ribeiro, J.A.; Sebastião, A.M. Modulation and metamodulation of synapses by adenosine. *Acta Physiol. (Oxf.),* **2010**, *199*(2), 161-169. [http://dx.doi.org/10.1111/j.1748-1716.2010.02115.x] [PMID: 20345418]

[17] Cunha, R.A. Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. *Purinergic Signal.,* **2005**, *1*(2), 111-134. [http://dx.doi.org/10.1007/s11302-005-0649-1] [PMID: 18404497]

[18] Hettinger, B.D.; Lee, A.; Linden, J.; Rosin, D.L. Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. *J. Comp. Neurol.,* **2001**, *431*(3), 331-346. [http://dx.doi.org/10.1002/1096-9861(20010312)431:3<331::AID-CNE1074>3.0.CO;2-W] [PMID: 11170009]

[19] Rebola, N.; Canas, P.M.; Oliveira, C.R.; Cunha, R.A. Different synaptic and subsynaptic localization of adenosine A2A receptors in the hippocampus and striatum of the rat. *Neuroscience,* **2005**, *132*(4), 893-903. [http://dx.doi.org/10.1016/j.neuroscience.2005.01.014] [PMID: 15857695]

[20] Ciruela, F.; Casadó, V.; Rodrigues, R.J.; Luján, R.; Burgueño, J.; Canals, M.; Borycz, J.; Rebola, N.; Goldberg, S.R.; Mallol, J.; Cortés, A.; Canela, E.I.; López-Giménez, J.F.; Milligan, G.; Lluis, C.; Cunha, R.A.; Ferré, S.; Franco, R. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. *J. Neurosci.,* **2006**, *26*(7), 2080-2087. [http://dx.doi.org/10.1523/JNEUROSCI.3574-05.2006] [PMID: 16481441]

[21] Ferré, S.; Agnati, L.F.; Ciruela, F.; Lluis, C.; Woods, A.S.; Fuxe, K.; Franco, R. Neurotransmitter receptor heteromers and their integrative role in 'local modules': the striatal spine module. *Brain Res. Brain Res. Rev.,* **2007**, *55*(1), 55-67. [http://dx.doi.org/10.1016/j.brainresrev.2007.01.007] [PMID: 17408563]

[22] Cabello, N.; Gandía, J.; Bertarelli, D.C.; Watanabe, M.; Lluís, C.; Franco, R.; Ferré, S.; Luján, R.; Ciruela, F. Metabotropic glutamate type 5, dopamine D2 and adenosine A2a receptors form higher-order oligomers in living cells. *J. Neurochem.,* **2009**, *109*(5), 1497-1507. [http://dx.doi.org/10.1111/j.1471-4159.2009.06078.x] [PMID: 19344374]

[23] Bogenpohl, J.W.; Ritter, S.L.; Hall, R.A.; Smith, Y. Adenosine A2A receptor in the monkey basal ganglia: ultrastructural localization and colocalization with the metabotropic glutamate receptor 5 in the striatum. *J. Comp. Neurol.,* **2012**, *520*(3), 570-589. [http://dx.doi.org/10.1002/cne.22751] [PMID: 21858817]

[24] Xie, J.D.; Chen, S.R.; Pan, H.L. Presynaptic mGluR5 receptor controls glutamatergic input through protein kinase C-NMDA receptors in paclitaxel-induced neuropathic pain. *J. Biol. Chem.,* **2017**, *292*(50), 20644-20654. [http://dx.doi.org/10.1074/jbc.M117.818476] [PMID: 29074619]

[25] Bragina, L.; Bonifacino, T.; Bassi, S.; Milanese, M.; Bonanno, G.; Conti, F. Differential expression of metabotropic glutamate and GABA receptors at neocortical glutamatergic and GABAergic axon terminals. *Front. Cell. Neurosci.,* **2015**, *9*, 345. [http://dx.doi.org/10.3389/fncel.2015.00345] [PMID: 26388733]

[26] Sheffler, D.J.; Gregory, K.J.; Rook, J.M.; Conn, P.J. Allosteric modulation of metabotropic glutamate receptors. *Adv. Pharmacol.,* **2011**, *62*, 37-77. [http://dx.doi.org/10.1016/B978-0-12-385952-5.00010-5] [PMID: 21907906]

[27] Rodrigues, R.J.; Alfaro, T.M.; Rebola, N.; Oliveira, C.R.; Cunha, R.A. Co-localization and functional interaction between adenosine A(2A) and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. *J. Neurochem.,* **2005**, *92*(3), 433-441. [http://dx.doi.org/10.1111/j.1471-4159.2004.02887.x] [PMID: 15659214]

[28] Ogata, T.; Nakamura, Y.; Schubert, P. Potentiated cAMP rise in metabotropically stimulated rat cultured astrocytes by a Ca^{2+}-related A1/A2 adenosine receptor cooperation. *Eur. J. Neurosci.,* **1996**, *8*(6), 1124-1131. [http://dx.doi.org/10.1111/j.1460-9568.1996.tb01280.x] [PMID: 8752582]

[29] Zhang, C.; Schmidt, J.T. Adenosine A1 and class II metabotropic glutamate receptors mediate shared presynaptic inhibition of retinotectal transmission. *J. Neurophysiol.,* **1999**, *82*(6), 2947-2955. [http://dx.doi.org/10.1152/jn.1999.82.6.2947] [PMID: 10601431]

[30] Ogata, T.; Nakamura, Y.; Tsuji, K.; Shibata, T.; Kataoka, K.; Schubert, P. Adenosine enhances intracellular Ca^{2+} mobilization in conjunction with metabotropic glutamate receptor activation by t-ACPD in cultured hippocampal astrocytes. *Neurosci. Lett.,* **1994**, *170*(1), 5-8. [http://dx.doi.org/10.1016/0304-3940(94)90225-9] [PMID: 8041512]

[31] Toms, N.J.; Roberts, P.J. Group 1 mGlu receptors elevate $[Ca^{2+}]i$ in rat cultured cortical type 2 astrocytes: $[Ca^{2+}]i$ synergy with adenosine A1 receptors. *Neuropharmacology,* **1999**, *38*(10), 1511-1517. [http://dx.doi.org/10.1016/S0028-3908(99)00090-8] [PMID: 10530813]

[32] Ciruela, F.; Escriche, M.; Burgueno, J.; Angulo, E.; Casado, V.; Soloviev, M.M.; Canela, E.I.; Mallol, J.; Chan, W.Y.; Lluis, C.; McIlhinney, R.A.; Franco, R. Metabotropic glutamate 1alpha and adenosine A1 receptors assemble into functionally interacting complexes. *J. Biol. Chem.,* **2001**, *276*(21), 18345-18351. [http://dx.doi.org/10.1074/jbc.M006960200] [PMID: 11278325]

[33] Kamikubo, Y.; Tabata, T.; Sakairi, H.; Hashimoto, Y.; Sakurai, T. Complex formation and functional interaction between adenosine A1 receptor and type-1 metabotropic glutamate receptor. *J. Pharmacol. Sci.,* **2015**, *128*(3), 125-130. [http://dx.doi.org/10.1016/j.jphs.2015.06.002] [PMID: 26154847]

[34] Albasanz, J.L.; León, D.; Ruíz, M.A.; Fernández, M.; Martín, M. Adenosine A1 receptor agonist treatment up-regulates rat brain metabotropic glutamate receptors. *Biochim. Biophys. Acta,* **2002**, *1593*(1), 69-75. [http://dx.doi.org/10.1016/S0167-4889(02)00330-0] [PMID: 12431785]

[35] León, D.A.; Albasanz, J.L.; Castillo, C.A.; Iglesias, I.; Martín, M. Effect of chronic gestational treatment with the adenosine A1 receptor agonist R-phenylisopropyladenosine on metabotropic glutamate receptors/phospholipase C pathway in maternal and fetal brain. *J. Neurosci. Res.,* **2008**, *86*(15), 3295-3305. [http://dx.doi.org/10.1002/jnr.21771] [PMID: 18615645]

[36] León, D.; Albasanz, J.L.; Castillo, C.A.; Martín, M. Effect of glutamate intake during gestation on adenosine A(1) receptor/adenylyl cyclase pathway in both maternal and fetal rat brain. *J. Neurochem.,* **2008**, *104*(2), 435-445. [PMID: 17953672]

[37] León, D.; Albasanz, J.L.; Ruíz, M.A.; Iglesias, I.; Martín, M. Effect of chronic gestational treatment with caffeine or theophylline on Group I metabotropic glutamate receptors in maternal and fetal brain. *J. Neurochem.,* **2005**, *94*(2), 440-451. [http://dx.doi.org/10.1111/j.1471-4159.2005.03211.x] [PMID: 15998294]

[38] Tabata, T.; Kawakami, D.; Hashimoto, K.; Kassai, H.; Yoshida, T.; Hashimotodani, Y.; Fredholm, B.B.; Sekino, Y.; Aiba, A.; Kano, M. G protein-independent neuromodulatory action of adenosine on metabotropic glutamate signalling in mouse cerebellar Purkinje cells. *J. Physiol.,* **2007**, *581*(Pt 2), 693-708. [http://dx.doi.org/10.1113/jphysiol.2007.129866] [PMID: 17379632]

[39] Kamikubo, Y.; Shimomura, T.; Fujita, Y.; Tabata, T.; Kashiyama, T.; Sakurai, T.; Fukurotani, K.; Kano, M. Functional cooperation of metabotropic adenosine and glutamate receptors regulates postsynaptic plasticity in the cerebellum. *J. Neurosci.,* **2013**, *33*(47), 18661-18671. [http://dx.doi.org/10.1523/JNEUROSCI.5567-12.2013] [PMID: 24259587]

[40] Budd, D.C.; Nicholls, D.G. Protein kinase C-mediated suppression of the presynaptic adenosine A1 receptor by a facilitatory metabotropic glutamate receptor. *J. Neurochem.,* **1995**, *65*(2), 615-621. [http://dx.doi.org/10.1046/j.1471-4159.1995.65020615.x] [PMID: 7616216]

[41] Shahraki, A.; Stone, T.W. Interactions between adenosine and metabotropic glutamate receptors in the rat hippocampal slice. *Br. J. Pharmacol.,* **2003**, *138*(6), 1059-1068. [http://dx.doi.org/10.1038/sj.bjp.0705083] [PMID: 12684261]

[42] Díaz-Cabiale, Z.; Vivó, M.; Del Arco, A.; O'Connor, W.T.; Harte, M.K.; Müller, C.E.; Martínez, E.; Popoli, P.; Fuxe, K.; Ferré, S. Metabotropic glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway in rats. Interactions with adenosine A(2A) and dopamine D(2) receptors. *Neurosci. Lett.,* **2002**, *324*(2), 154-158. [http://dx.doi.org/10.1016/S0304-3940(02)00179-9] [PMID: 11988350]

[43] Nishi, A.; Liu, F.; Matsuyama, S.; Hamada, M.; Higashi, H.; Nairn, A.C.; Greengard, P. Metabotropic mGlu5 receptors regulate adenosine A2A receptor signaling. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*(3), 1322-1327. [http://dx.doi.org/10.1073/pnas.0237126100] [PMID: 12538871]

[44] Ferré, S.; Karcz-Kubicha, M.; Hope, B.T.; Popoli, P.; Burgueño, J.; Gutiérrez, M.A.; Casadó, V.; Fuxe, K.; Goldberg, S.R.; Lluis, C.; Franco, R.; Ciruela, F. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*(18), 11940-11945. [http://dx.doi.org/10.1073/pnas.172393799] [PMID: 12189203]

[45] Domenici, M.R.; Pepponi, R.; Martire, A.; Tebano, M.T.; Potenza, R.L.; Popoli, P. Permissive role of adenosine A2A receptors on metabotropic glutamate receptor 5 (mGluR5)-mediated effects in the striatum. *J. Neurochem.,* **2004**, *90*(5), 1276-1279. [http://dx.doi.org/10.1111/j.1471-4159.2004.02607.x] [PMID: 15312183]

[46] Tebano, M.T.; Martire, A.; Pepponi, R.; Domenici, M.R.; Popoli, P. Is the functional interaction between adenosine A(2A) receptors and metabotropic glutamate 5 receptors a general mechanism in the brain? Differences and similarities between the striatum and the hippocampus. *Purinergic Signal.,* **2006**, *2*(4), 619-625. [http://dx.doi.org/10.1007/s11302-006-9026-y] [PMID: 18404464]

[47] Beggiato, S.; Tomasini, M.C.; Borelli, A.C.; Borroto-Escuela, D.O.; Fuxe, K.; Antonelli, T.; Tanganelli, S.; Ferraro, L. Functional role of striatal A2A, D2, and mGlu5 receptor interactions in regulating striatopallidal GABA neuronal transmission. *J. Neurochem.,* **2016**, *138*(2), 254-264. [http://dx.doi.org/10.1111/jnc.13652] [PMID: 27127992]

[48] Popoli, P.; Pèzzola, A.; Torvinen, M.; Reggio, R.; Pintor, A.; Scarchilli, L.; Fuxe, K.; Ferré, S. The selective mGlu(5) receptor ago-

nist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: interactions with adenosine A(2a) receptors. *Neuropsychopharmacology,* **2001**, *25*(4), 505-513. [http://dx.doi.org/10.1016/S0893-133X(01)00256-1] [PMID: 11557164]

[49] Coccurello, R.; Breysse, N.; Amalric, M. Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. *Neuropsychopharmacology,* **2004**, *29*(8), 1451-1461. [http://dx.doi.org/10.1038/sj.npp.1300444] [PMID: 15039773]

[50] Kachroo, A.; Orlando, L.R.; Grandy, D.K.; Chen, J.F.; Young, A.B.; Schwarzschild, M.A. Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. *J. Neurosci.,* **2005**, *25*(45), 10414-10419. [http://dx.doi.org/10.1523/JNEUROSCI.3660-05.2005] [PMID: 16280580]

[51] Brown, R.M.; Duncan, J.R.; Stagnitti, M.R.; Ledent, C.; Lawrence, A.J. mGlu5 and adenosine A2A receptor interactions regulate the conditioned effects of cocaine. *Int. J. Neuropsychopharmacol.,* **2012**, *15*(7), 995-1001. [http://dx.doi.org/10.1017/S146114571100126X] [PMID: 21816123]

[52] Adams, C.L.; Cowen, M.S.; Short, J.L.; Lawrence, A.J. Combined antagonism of glutamate mGlu5 and adenosine A2A receptors interact to regulate alcohol-seeking in rats. *Int. J. Neuropsychopharmacol.,* **2008**, *11*(2), 229-241. [http://dx.doi.org/10.1017/S1461145707007845] [PMID: 17517168]

[53] Wright, S.R.; Zanos, P.; Georgiou, P.; Yoo, J.H.; Ledent, C.; Hourani, S.M.; Kitchen, I.; Winsky-Sommerer, R.; Bailey, A. A critical role of striatal A2A R-mGlu5 R interactions in modulating the psychomotor and drug-seeking effects of methamphetamine. *Addict. Biol.,* **2016**, *21*(4), 811-825. [http://dx.doi.org/10.1111/adb.12259] [PMID: 25975203]

[54] De Las Rivas, J.; Fontanillo, C. Protein-protein interactions essentials: key concepts to building and analyzing interactome networks. *PLOS Comput. Biol.,* **2010**, *6*(6), e1000807. [http://dx.doi.org/10.1371/journal.pcbi.1000807] [PMID: 20589078]

[55] Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.,* **2003**, *13*(11), 2498-2504. [http://dx.doi.org/10.1101/gr.1239303] [PMID: 14597658]

[56] Berman, H.; Henrick, K.; Nakamura, H. Announcing the worldwide protein data bank. *Nat. Struct. Biol.,* **2003**, *10*(12), 980. [http://dx.doi.org/10.1038/nsb1203-980] [PMID: 14634627]

[57] Guo, H.; An, S.; Ward, R.; Yang, Y.; Liu, Y.; Guo, X.X.; Hao, Q.; Xu, T.R. Methods used to study the oligomeric structure of G-protein-coupled receptors. *Biosci. Rep.,* **2017**, *37*(2), BSR20160547. [http://dx.doi.org/10.1042/BSR20160547] [PMID: 28062602]

[58] Lohse, M.J. Dimerization in GPCR mobility and signaling. *Curr. Opin. Pharmacol.,* **2010**, *10*(1), 53-58. [http://dx.doi.org/10.1016/j.coph.2009.10.007] [PMID: 19910252]

[59] Sevastyanova, T.N.; Kammermeier, P.J. Cooperative signaling between homodimers of metabotropic glutamate receptors 1 and 5. *Mol. Pharmacol.,* **2014**, *86*(5), 492-504. [http://dx.doi.org/10.1124/mol.114.093468] [PMID: 25113912]

[60] Schonenbach, N.S.; Rieth, M.D.; Han, S.; O'Malley, M.A. Adenosine A2a receptors form distinct oligomers in protein detergent complexes. *FEBS Lett.,* **2016**, *590*(18), 3295-3306. [http://dx.doi.org/10.1002/1873-3468.12367] [PMID: 27543907]

[61] El Moustaine, D.; Granier, S.; Doumazane, E.; Scholler, P.; Rahmeh, R.; Bron, P.; Mouillac, B.; Banères, J.L.; Rondard, P.; Pin, J.P. Distinct roles of metabotropic glutamate receptor dimerization in agonist activation and G-protein coupling. *Proc. Natl. Acad. Sci. USA,* **2012**, *109*(40), 16342-16347. [http://dx.doi.org/10.1073/pnas.1205838109] [PMID: 22988116]

[62] Maurel, D.; Comps-Agrar, L.; Brock, C.; Rives, M.L.; Bourrier, E.; Ayoub, M.A.; Bazin, H.; Tinel, N.; Durroux, T.; Prézeau, L.; Trinquet, E.; Pin, J.P. Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. *Nat. Methods,* **2008**, *5*(6), 561-567. [http://dx.doi.org/10.1038/nmeth.1213] [PMID: 18488035]

[63] Doumazane, E.; Scholler, P.; Zwier, J.M.; Trinquet, E.; Rondard, P.; Pin, J.P. A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. *FASEB J.,* **2011**, *25*(1), 66-77. [http://dx.doi.org/10.1096/fj.10-163147] [PMID: 20826542]

[64] Levitz, J.; Habrian, C.; Bharill, S.; Fu, Z.; Vafabakhsh, R.; Isacoff, E.Y. Mechanism of assembly and cooperativity of homomeric and heteromeric metabotropic glutamate receptors. *Neuron,* **2016**, *92*(1), 143-159. [http://dx.doi.org/10.1016/j.neuron.2016.08.036] [PMID: 27641494]

[65] Xue, L.; Rovira, X.; Scholler, P.; Zhao, H.; Liu, J.; Pin, J.P.; Rondard, P. Major ligand-induced rearrangement of the heptahelical domain interface in a GPCR dimer. *Nat. Chem. Biol.,* **2015**, *11*(2), 134-140. [http://dx.doi.org/10.1038/nchembio.1711] [PMID: 25503927]

[66] Ciruela, F.; Fernández-Dueñas, V.; Llorente, J.; Borroto-Escuela, D.; Cuffi, M.L.; Carbonell, L.; Sánchez, S.; Agnati, L.F.; Fuxe, K.; Tasca, C.I. G protein-coupled receptor oligomerization and brain integration: focus on adenosinergic transmission. *Brain Res.,* **2012**, *1476*, 86-95. [http://dx.doi.org/10.1016/j.brainres.2012.04.056] [PMID: 22575562]

[67] Cabrera-Vera, T.M.; Vanhauwe, J.; Thomas, T.O.; Medkova, M.; Preininger, A.; Mazzoni, M.R.; Hamm, H.E. Insights into G protein structure, function, and regulation. *Endocr. Rev.,* **2003**, *24*(6), 765-781. [http://dx.doi.org/10.1210/er.2000-0026] [PMID: 14671004]

[68] Gomeza, J.; Mary, S.; Brabet, I.; Parmentier, M.L.; Restituito, S.; Bockaert, J.; Pin, J.P. Coupling of metabotropic glutamate receptors 2 and 4 to G alpha 15, G alpha 16, and chimeric G alpha q/i proteins: characterization of new antagonists. *Mol. Pharmacol.,* **1996**, *50*(4), 923-930. [PMID: 8863838]

[69] Dhingra, A.; Lyubarsky, A.; Jiang, M.; Pugh, E.N., Jr; Birnbaumer, L.; Sterling, P.; Vardi, N. The light response of ON bipolar neurons requires G[alpha]o. *J. Neurosci.,* **2000**, *20*(24), 9053-9058. [http://dx.doi.org/10.1523/JNEUROSCI.20-24-09053.2000] [PMID: 11124982]

[70] Waldhoer, M.; Wise, A.; Milligan, G.; Freissmuth, M.; Nanoff, C. Kinetics of ternary complex formation with fusion proteins composed of the A(1)-adenosine receptor and G protein alpha-subunits. *J. Biol. Chem.,* **1999**, *274*(43), 30571-30579. [http://dx.doi.org/10.1074/jbc.274.43.30571] [PMID: 10521440]

[71] Soundararajan, M.; Willard, F.S.; Kimple, A.J.; Turnbull, A.P.; Ball, L.J.; Schoch, G.A.; Gileadi, C.; Fedorov, O.Y.; Dowler, E.F.; Higman, V.A.; Hutsell, S.Q.; Sundström, M.; Doyle, D.A.; Siderovski, D.P. Structural diversity in the RGS domain and its interaction with heterotrimeric G protein alpha-subunits. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(17), 6457-6462. [http://dx.doi.org/10.1073/pnas.0801508105] [PMID: 18434541]

[72] Snow, B.E.; Hall, R.A.; Krumins, A.M.; Brothers, G.M.; Bouchard, D.; Brothers, C.A.; Chung, S.; Mangion, J.; Gilman, A.G.; Lefkowitz, R.J.; Siderovski, D.P. GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. *J. Biol. Chem.,* **1998**, *273*(28), 17749-17755. [http://dx.doi.org/10.1074/jbc.273.28.17749] [PMID: 9651375]

[73] Roux, B.T.; Cottrell, G.S. G protein-coupled receptors: what a difference a 'partner' makes. *Int. J. Mol. Sci.,* **2014**, *15*(1), 1112-1142. [http://dx.doi.org/10.3390/ijms15011112] [PMID: 24441568]

[74] Gracia, E.; Farré, D.; Cortés, A.; Ferrer-Costa, C.; Orozco, M.; Mallol, J.; Lluís, C.; Canela, E.I.; McCormick, P.J.; Franco, R.; Fanelli, F.; Casadó, V. The catalytic site structural gate of adenosine deaminase allosterically modulates ligand binding to adenosine receptors. *FASEB J.,* **2013**, *27*(3), 1048-1061. [http://dx.doi.org/10.1096/fj.12-212621] [PMID: 23193172]

[75] Franco, R.; Casadó, V.; Ciruela, F.; Saura, C.; Mallol, J.; Canela, E.I.; Lluis, C. Cell surface adenosine deaminase: much more than an ectoenzyme. *Prog. Neurobiol.,* **1997**, *52*(4), 283-294. [http://dx.doi.org/10.1016/S0301-0082(97)00013-0] [PMID: 9247966]

[76] Torvinen, M.; Ginés, S.; Hillion, J.; Latini, S.; Canals, M.; Ciruela, F.; Bordoni, F.; Staines, W.; Pedata, F.; Agnati, L.F.; Lluis, C.; Franco, R.; Ferré, S.; Fuxe, K. Interactions among adenosine deaminase, adenosine A(1) receptors and dopamine D(1) receptors in stably cotransfected fibroblast cells and neurons. *Neuroscience,*

2002, *113*(3), 709-719. [http://dx.doi.org/10.1016/S0306-4522(02)00058-1] [PMID: 12150791]

[77] Herrera, C.; Casadó, V.; Ciruela, F.; Schofield, P.; Mallol, J.; Lluis, C.; Franco, R. Adenosine A2B receptors behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and cultured cells. *Mol. Pharmacol.,* **2001**, *59*(1), 127-134. [http://dx.doi.org/10.1124/mol.59.1.127] [PMID: 11125033]

[78] Málaga-Diéguez, L.; Yang, Q.; Bauer, J.; Pankevych, H.; Freissmuth, M.; Nanoff, C. Pharmacochaperoning of the A1 adenosine receptor is contingent on the endoplasmic reticulum. *Mol. Pharmacol.,* **2010**, *77*(6), 940-952. [http://dx.doi.org/10.1124/mol.110.063511] [PMID: 20219842]

[79] Brakeman, P.R.; Lanahan, A.A.; O'Brien, R.; Roche, K.; Barnes, C.A.; Huganir, R.L.; Worley, P.F. Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature,* **1997**, *386*(6622), 284-288. [http://dx.doi.org/10.1038/386284a0] [PMID: 9069287]

[80] Ango, F.; Prézeau, L.; Muller, T.; Tu, J.C.; Xiao, B.; Worley, P.F.; Pin, J.P.; Bockaert, J.; Fagni, L. Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein Homer. *Nature,* **2001**, *411*(6840), 962-965. [http://dx.doi.org/10.1038/35082096] [PMID: 11418862]

[81] Ango, F.; Robbe, D.; Tu, J.C.; Xiao, B.; Worley, P.F.; Pin, J.P.; Bockaert, J.; Fagni, L. Homer-dependent cell surface expression of metabotropic glutamate receptor type 5 in neurons. *Mol. Cell. Neurosci.,* **2002**, *20*(2), 323-329. [http://dx.doi.org/10.1006/mcne.2002.1100] [PMID: 12093163]

[82] Paquet, M.; Asay, M.J.; Fam, S.R.; Inuzuka, H.; Castleberry, A.M.; Oller, H.; Smith, Y.; Yun, C.C.; Traynelis, S.F.; Hall, R.A. The PDZ scaffold NHERF-2 interacts with mGluR5 and regulates receptor activity. *J. Biol. Chem.,* **2006**, *281*(40), 29949-29961. [http://dx.doi.org/10.1074/jbc.M602262200] [PMID: 16891310]

[83] Ritter-Makinson, S.L.; Paquet, M.; Bogenpohl, J.W.; Rodin, R.E.; Chris Yun, C.; Weinman, E.J.; Smith, Y.; Hall, R.A. Group II metabotropic glutamate receptor interactions with NHERF scaffold proteins: Implications for receptor localization in brain. *Neuroscience,* **2017**, *353*, 58-75. [http://dx.doi.org/10.1016/j.neuroscience.2017.03.060] [PMID: 28392297]

[84] Enz, R. The actin-binding protein Filamin-A interacts with the metabotropic glutamate receptor type 7. *FEBS Lett.,* **2002**, *514*(2-3), 184-188. [http://dx.doi.org/10.1016/S0014-5793(02)02361-X] [PMID: 11943148]

[85] Canela, L.; Luján, R.; Lluís, C.; Burgueño, J.; Mallol, J.; Canela, E.I.; Franco, R.; Ciruela, F. The neuronal $Ca^{(2+)}$ -binding protein 2 (NECAB2) interacts with the adenosine A(2A) receptor and modulates the cell surface expression and function of the receptor. *Mol. Cell. Neurosci.,* **2007**, *36*(1), 1-12. [http://dx.doi.org/10.1016/j.mcn.2007.05.007] [PMID: 17689978]

[86] Canela, L.; Fernández-Dueñas, V.; Albergaria, C.; Watanabe, M.; Lluís, C.; Mallol, J.; Canela, E.I.; Franco, R.; Luján, R.; Ciruela, F. The association of metabotropic glutamate receptor type 5 with the neuronal Ca^{2+}-binding protein 2 modulates receptor function. *J. Neurochem.,* **2009**, *111*(2), 555-567. [http://dx.doi.org/10.1111/j.1471-4159.2009.06348.x] [PMID: 19694902]

[87] Uematsu, K.; Heiman, M.; Zelenina, M.; Padovan, J.; Chait, B.T.; Aperia, A.; Nishi, A.; Greengard, P. Protein kinase A directly phosphorylates metabotropic glutamate receptor 5 to modulate its function. *J. Neurochem.,* **2015**, *132*(6), 677-686. [http://dx.doi.org/10.1111/jnc.13038] [PMID: 25639954]

[88] Palmer, T.M.; Stiles, G.L. Stimulation of A(2A) adenosine receptor phosphorylation by protein kinase C activation: evidence for regulation by multiple protein kinase C isoforms. *Biochemistry,* **1999**, *38*(45), 14833-14842. [http://dx.doi.org/10.1021/bi990825p] [PMID: 10555965]

[89] Cartmell, J.; Goepfert, F.; Knoflach, F.; Pink, J.R.; Bleuel, Z.; Richards, J.G.; Schaffhauser, H.; Kemp, J.A.; Wichmann, J.; Mutel, V. Effect of metabotropic glutamate receptor activation on receptor-mediated cyclic AMP responses in primary cultures of rat striatal neurones. *Brain Res.,* **1998**, *791*(1-2), 191-199. [http://dx.doi.org/10.1016/S0006-8993(98)00094-8] [PMID: 9593890]

[90] de Mendonça, A.; Ribeiro, J.A. Influence of metabotropic glutamate receptor agonists on the inhibitory effects of adenosine A1 receptor activation in the rat hippocampus. *Br. J. Pharmacol.,* **1997**, *121*(8), 1541-1548. [http://dx.doi.org/10.1038/sj.bjp.0701291] [PMID: 9283686]

[91] Macek, T.A.; Schaffhauser, H.; Conn, P.J. Protein kinase C and A3 adenosine receptor activation inhibit presynaptic metabotropic glutamate receptor (mGluR) function and uncouple mGluRs from GTP-binding proteins. *J. Neurosci.,* **1998**, *18*(16), 6138-6146. [http://dx.doi.org/10.1523/JNEUROSCI.18-16-06138.1998] [PMID: 9698308]

[92] Minakami, R.; Jinnai, N.; Sugiyama, H. Phosphorylation and calmodulin binding of the metabotropic glutamate receptor subtype 5 (mGluR5) are antagonistic *in vitro*. *J. Biol. Chem.,* **1997**, *272*(32), 20291-20298. [http://dx.doi.org/10.1074/jbc.272.32.20291] [PMID: 9242710]

[93] Saugstad, J.A.; Yang, S.; Pohl, J.; Hall, R.A.; Conn, P.J. Interaction between metabotropic glutamate receptor 7 and alpha tubulin. *J. Neurochem.,* **2002**, *80*(6), 980-988. [http://dx.doi.org/10.1046/j.0022-3042.2002.00778.x] [PMID: 11953448]

[94] Lidwell, K.; Dillon, J.; Sihota, A.; O'Connor, V.; Pilkington, B. Determining calmodulin binding to metabotropic glutamate receptors with distinct protein-interaction methods. *Biochem. Soc. Trans.,* **2004**, *32*(Pt 5), 868-870. [http://dx.doi.org/10.1042/BST0320868] [PMID: 15494036]

[95] El Far, O.; Betz, H. G-protein-coupled receptors for neurotransmitter amino acids: C-terminal tails, crowded signalosomes. *Biochem. J.,* **2002**, *365*(Pt 2), 329-336. [http://dx.doi.org/10.1042/bj20020481] [PMID: 12006104]

[96] Ko, S.J.; Isozaki, K.; Kim, I.; Lee, J.H.; Cho, H.J.; Sohn, S.Y.; Oh, S.R.; Park, S.; Kim, D.G.; Kim, C.H.; Roche, K.W. PKC phosphorylation regulates mGluR5 trafficking by enhancing binding of Siah-1A. *J. Neurosci.,* **2012**, *32*(46), 16391-16401. [http://dx.doi.org/10.1523/JNEUROSCI.1964-12.2012] [PMID: 23152621]

[97] Nakajima, Y.; Yamamoto, T.; Nakayama, T.; Nakanishi, S. A relationship between protein kinase C phosphorylation and calmodulin binding to the metabotropic glutamate receptor subtype 7. *J. Biol. Chem.,* **1999**, *274*(39), 27573-27577. [http://dx.doi.org/10.1074/jbc.274.39.27573] [PMID: 10488094]

[98] El Far, O.; Bofill-Cardona, E.; Airas, J.M.; O'Connor, V.; Boehm, S.; Freissmuth, M.; Nanoff, C.; Betz, H. Mapping of calmodulin and Gbetagamma binding domains within the C-terminal region of the metabotropic glutamate receptor 7A. *J. Biol. Chem.,* **2001**, *276*(33), 30662-30669. [http://dx.doi.org/10.1074/jbc.M102573200] [PMID: 11395497]

[99] Sorensen, S.D.; Macek, T.A.; Cai, Z.; Saugstad, J.A.; Conn, P.J. Dissociation of protein kinase-mediated regulation of metabotropic glutamate receptor 7 (mGluR7) interactions with calmodulin and regulation of mGluR7 function. *Mol. Pharmacol.,* **2002**, *61*(6), 1303-1312. [http://dx.doi.org/10.1124/mol.61.6.1303] [PMID: 12021391]

[100] Navarro, G.; Aymerich, M.S.; Marcellino, D.; Cortés, A.; Casadó, V.; Mallol, J.; Canela, E.I.; Agnati, L.; Woods, A.S.; Fuxe, K.; Lluís, C.; Lanciego, J.L.; Ferré, S.; Franco, R. Interactions between calmodulin, adenosine A2A, and dopamine D2 receptors. *J. Biol. Chem.,* **2009**, *284*(41), 28058-28068. [http://dx.doi.org/10.1074/jbc.M109.034231] [PMID: 19632986]

[101] Kitano, J.; Kimura, K.; Yamazaki, Y.; Soda, T.; Shigemoto, R.; Nakajima, Y.; Nakanishi, S. Tamalin, a PDZ domain-containing protein, links a protein complex formation of group 1 metabotropic glutamate receptors and the guanine nucleotide exchange factor cytohesins. *J. Neurosci.,* **2002**, *22*(4), 1280-1289. [http://dx.doi.org/10.1523/JNEUROSCI.22-04-01280.2002] [PMID: 11850456]

[102] Gsandtner, I.; Charalambous, C.; Stefan, E.; Ogris, E.; Freissmuth, M.; Zezula, J. Heterotrimeric G protein-independent signaling of a G protein-coupled receptor. Direct binding of ARNO/cytohesin-2 to the carboxyl terminus of the A2A adenosine receptor is necessary for sustained activation of the ERK/MAP kinase pathway. *J. Biol. Chem.,* **2005**, *280*(36), 31898-31905. [http://dx.doi.org/10.1074/jbc.M506515200] [PMID: 16027149]

[103] Francesconi, A.; Duvoisin, R.M. Opposing effects of protein kinase C and protein kinase A on metabotropic glutamate receptor signaling: selective desensitization of the inositol trisphosphate/Ca^{2+} pathway by phosphorylation of the receptor-G protein-coupling

domain. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(11), 6185-6190. [http://dx.doi.org/10.1073/pnas.97.11.6185] [PMID: 10823959]

[104] Dev, K.K.; Nakajima, Y.; Kitano, J.; Braithwaite, S.P.; Henley, J.M.; Nakanishi, S. PICK1 interacts with and regulates PKC phosphorylation of mGLUR7. *J. Neurosci.,* **2000**, *20*(19), 7252-7257. [http://dx.doi.org/10.1523/JNEUROSCI.20-19-07252.2000] [PMID: 11007882]

[105] Dev, K.K.; Nakanishi, S.; Henley, J.M. The PDZ domain of PICK1 differentially accepts protein kinase C-alpha and GluR2 as interacting ligands. *J. Biol. Chem.,* **2004**, *279*(40), 41393-41397. [http://dx.doi.org/10.1074/jbc.M404499200] [PMID: 15247289]

[106] Hirbec, H.; Perestenko, O.; Nishimune, A.; Meyer, G.; Nakanishi, S.; Henley, J.M.; Dev, K.K. The PDZ proteins PICK1, GRIP, and syntenin bind multiple glutamate receptor subtypes. Analysis of PDZ binding motifs. *J. Biol. Chem.,* **2002**, *277*(18), 15221-15224. [http://dx.doi.org/10.1074/jbc.C200112200] [PMID: 11891216]

[107] Boudin, H.; Doan, A.; Xia, J.; Shigemoto, R.; Huganir, R.L.; Worley, P.; Craig, A.M. Presynaptic clustering of mGluR7a requires the PICK1 PDZ domain binding site. *Neuron,* **2000**, *28*(2), 485-497. [http://dx.doi.org/10.1016/S0896-6273(00)00127-6] [PMID: 11144358]

[108] Thompson, J.W.; Nagel, J.; Hoving, S.; Gerrits, B.; Bauer, A.; Thomas, J.R.; Kirschner, M.W.; Schirle, M.; Luchansky, S.J. Quantitative Lys-ϵ-Gly-Gly (diGly) proteomics coupled with inducible RNAi reveals ubiquitin-mediated proteolysis of DNA damage-inducible transcript 4 (DDIT4) by the E3 ligase HUWE1. *J. Biol. Chem.,* **2014**, *289*(42), 28942-28955. [http://dx.doi.org/10.1074/jbc.M114.573352] [PMID: 25147182]

[109] Wagner, S. A.; Beli, P.; Weinert, B. T.; Nielsen, M. L.; Cox, J.; Mann, M.; Choudhary, C. A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics,* **2011**, *10*(10), M111-013284.

[110] Danielsen, J. M.; Sylvestersen, K. B.; Bekker-Jensen, S.; Szklarczyk, D.; Poulsen, J. W.; Horn, H.; Jensen, L. J.; Mailand, N.; Nielsen, M. L. Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. *Mol. Cell Proteom.,* **2011**, *10*(3), M110-003590. [http://dx.doi.org/10.1074/mcp.M110.003590]

[111] Dores, M.R.; Trejo, J. Atypical regulation of G protein-coupled receptor intracellular trafficking by ubiquitination. *Curr. Opin. Cell Biol.,* **2014**, *27*, 44-50. [http://dx.doi.org/10.1016/j.ceb.2013.11.004] [PMID: 24680429]

[112] Whistler, J.L.; Enquist, J.; Marley, A.; Fong, J.; Gladher, F.; Tsuruda, P.; Murray, S.R.; Von Zastrow, M. Modulation of postendocytic sorting of G protein-coupled receptors. *Science,* **2002**, *297*(5581), 615-620. [http://dx.doi.org/10.1126/science.1073308] [PMID: 12142540]

[113] Heydorn, A.; Søndergaard, B.P.; Ersbøll, B.; Holst, B.; Nielsen, F.C.; Haft, C.R.; Whistler, J.; Schwartz, T.W. A library of 7TM receptor C-terminal tails. Interactions with the proposed post-endocytic sorting proteins ERM-binding phosphoprotein 50 (EBP50), N-ethylmaleimide-sensitive factor (NSF), sorting nexin 1 (SNX1), and G protein-coupled receptor-associated sorting protein (GASP). *J. Biol. Chem.,* **2004**, *279*(52), 54291-54303. [http://dx.doi.org/10.1074/jbc.M406169200] [PMID: 15452121]

[114] Schonenbach, N.S.; Hussain, S.; O'Malley, M.A. Structure and function of G protein-coupled receptor oligomers: implications for drug discovery. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.,* **2015**, *7*(3), 408-427. [http://dx.doi.org/10.1002/wnan.1319] [PMID: 25521522]

[115] Ferré, S.; Casadó, V.; Devi, L.A.; Filizola, M.; Jockers, R.; Lohse, M.J.; Milligan, G.; Pin, J.P.; Guitart, X. G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol. Rev.,* **2014**, *66*(2), 413-434. [http://dx.doi.org/10.1124/pr.113.008052] [PMID: 24515647]

[116] Gracia, E.; Moreno, E.; Cortés, A.; Lluís, C.; Mallol, J.; McCormick, P.J.; Canela, E.I.; Casadó, V. Homodimerization of adenosine A_1 receptors in brain cortex explains the biphasic effects of caffeine. *Neuropharmacology,* **2013**, *71*, 56-69. [http://dx.doi.org/10.1016/j.neuropharm.2013.03.005] [PMID: 23523559]

[117] Ferre, S.; von Euler, G.; Johansson, B.; Fredholm, B.B.; Fuxe, K. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. USA,* **1991**, *88*(16), 7238-7241. [http://dx.doi.org/10.1073/pnas.88.16.7238] [PMID: 1678519]

[118] Kühhorn, J.; Hübner, H.; Gmeiner, P. Bivalent dopamine D2 receptor ligands: synthesis and binding properties. *J. Med. Chem.,* **2011**, *54*(13), 4896-4903. [http://dx.doi.org/10.1021/jm2004859] [PMID: 21599022]

[119] Borroto-Escuela, D.O.; Pintsuk, J.; Schäfer, T.; Friedland, K.; Ferraro, L.; Tanganelli, S.; Liu, F.; Fuxe, K. Multiple D2 heteroreceptor complexes: new targets for treatment of schizophrenia. *Ther. Adv. Psychopharmacol.,* **2016**, *6*(2), 77-94. [http://dx.doi.org/10.1177/2045125316637570] [PMID: 27141290]

[120] Shen, J.; Zhang, L.; Song, W.L.; Meng, T.; Wang, X.; Chen, L.; Feng, L.Y.; Xu, Y.C.; Shen, J.K. Design, synthesis and biological evaluation of bivalent ligands against A(1)-D(1) receptor heteromers. *Acta Pharmacol. Sin.,* **2013**, *34*(3), 441-452. [http://dx.doi.org/10.1038/aps.2012.151] [PMID: 23334237]

[121] Soriano, A.; Ventura, R.; Molero, A.; Hoen, R.; Casadó, V.; Cortés, A.; Fanelli, F.; Albericio, F.; Lluís, C.; Franco, R.; Royo, M. Adenosine A2A receptor-antagonist/dopamine D2 receptor-agonist bivalent ligands as pharmacological tools to detect A2A-D2 receptor heteromers. *J. Med. Chem.,* **2009**, *52*(18), 5590-5602. [http://dx.doi.org/10.1021/jm900298c] [PMID: 19711895]

[122] Peterson, C.D.; Kitto, K.F.; Akgün, E.; Lunzer, M.M.; Riedl, M.S.; Vulchanova, L.; Wilcox, G.L.; Portoghese, P.S.; Fairbanks, C.A. Bivalent ligand that activates mu opioid receptor and antagonizes mGluR5 receptor reduces neuropathic pain in mice. *Pain,* **2017**, *158*(12), 2431-2441. [http://dx.doi.org/10.1097/j.pain.0000000000001050] [PMID: 28891868]

[123] Hasbi, A.; O'Dowd, B.F.; George, S.R. Dopamine D1-D2 receptor heteromer signaling pathway in the brain: emerging physiological relevance. *Mol. Brain,* **2011**, *4*, 26. [http://dx.doi.org/10.1186/1756-6606-4-26] [PMID: 21663703]

[124] Bhattacharyya, S. Inside story of Group I Metabotropic Glutamate Receptors (mGluRs). *Int. J. Biochem. Cell Biol.,* **2016**, *77*(Pt B), 205-12.

[125] Cordomí, A.; Navarro, G.; Aymerich, M.S.; Franco, R. Structures for G-protein-coupled receptor tetramers in complex with G Proteins. *Trends Biochem. Sci.,* **2015**, *40*(10), 548-551. [http://dx.doi.org/10.1016/j.tibs.2015.07.007] [PMID: 26410595]

[126] Navarro, G.; Cordomí, A.; Zelman-Femiak, M.; Brugarolas, M.; Moreno, E.; Aguinaga, D.; Perez-Benito, L.; Cortés, A.; Casadó, V.; Mallol, J.; Canela, E.I.; Lluís, C.; Pardo, L.; García-Sáez, A.J.; McCormick, P.J.; Franco, R. Quaternary structure of a G-protein-coupled receptor heterotetramer in complex with Gi and Gs. *BMC Biol.,* **2016**, *14*, 26. [http://dx.doi.org/10.1186/s12915-016-0247-4] [PMID: 27048449]