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Metabotropic Receptors for Glutamate and GABA

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1. Introduction

G protein-coupled receptors (GPCRs) are the largest superfamily of transmembrane proteins and due to their ubiquitous expression and vast array of functions they present attractive targets for the treatment of a wide number of diseases and disorders. Accordingly, they represent up to 30% of targets of current therapeutics (Overington et al., 2006). Despite the capacity of GPCRs to modulate many (patho-)physiological functions there is a high attrition rate with regard to new compounds entering clinical trials. There are many reasons for the number of failed drug-like compounds such as non-specificity, unfavourable pharmacokinetic profile and lack of clinical efficacy. In this regard, molecules targeting neurotransmitter receptors in the CNS traditionally have poor side-effect profiles due to the high concentrations required to pass the blood-brain barrier. There remain many specific challenges in drug discovery such as promiscuous GPCR-effector coupling; differential cell- and tissue-specific effects; ligand-induced changes in receptor trafficking; and protein-protein interactions and receptor oligomerisation (Galandrin et al., 2007; Hanyaloglu and von Zastrow, 2008; Kniazeff et al., 2011; Wettschureck and Offermanns, 2005).

GPCRs are divided into three main classes (A-C) based on structural homology; however all GPCRs possess a 7-alpha-helical transmembrane-spanning (7TM) domain, which facilitates the transduction of extracellular signals into intracellular responses. GPCRs recognise a myriad of different stimuli from photons, amino acids and biogenic amines to large peptides and proteins. Class A (rhodopsin-like) GPCRs are among the best characterised and consist of a relatively short N-terminal domain, a 7TM domain connected by extracellular and intracellular loops, and an intracellular C-terminal domain (Fredriksson et al., 2003). Class B (secretin-like) GPCRs have comparatively long N-terminal domains with similar 7TM and C-terminal topography as Class A receptors. By far and away, Class C (glutamate-like) GPCRs have the most distinct topography compared the other GPCRs; they possess large, structured N-terminal domains, which form a venus-fly trap-like structure known as the venus-fly trap (VFT) domain. The VFT domain is often (with exceptions) connected to the 7TM domain via a cysteine-rich domain, and further to this the C-terminal domain is often comparatively longer than those of Class A GPCRs. Structurally, all GPCRs are similar in their 7TM domains, yet the activation mechanisms, at least by the endogenous ligand varies

greatly across the classes. The orthosteric (endogenous ligand) binding site in Class A GPCRs lies in the 7TM helical bundle (with exceptions, e.g. CXCR4 chemokine receptor and relaxin family receptors (Allen et al., 2007; Sudo et al., 2003)); class B receptor ligands tend to bind in the large N-terminal domain and have been postulated to possess a bimodal receptor activation mechanism, whereby after the ligand binding event the ligand-N-terminal complex inserts into the 7TM helical bundle to elicit receptor activation (Hoare, 2005); class C receptor orthosteric ligands bind in the VFT domain and, through a series of conformational changes, are able to induce receptor activation via the 7TM domain (Pin et al., 2004)(Figure 1).



Fig. 1. Canonical orthosteric ligand-binding domains of the three classes of GPCRs. Highlighted in yellow are the typical binding regions of orthosteric ligands, in addition to the general architecture of the three major classes of GPCRs.

One large hindrance to drug discovery is the high degree of protein sequence and structural conservation between orthosteric sites of receptors of the same family, increasing the difficulty to specifically and selectively target a single receptor subtype. However, by their very nature GPCRs are highly dynamic proteins that are able to adopt a spectrum of conformational arrangements and it is this characteristic that allows GPCRs to be modulated by, not only a range of orthosteric ligands, but also ligands that bind in a topographically distinct region to the orthosteric binding pocket. These ligands are known as allosteric ligands and are able to modulate the affinity and/or efficacy of the orthosteric ligand, and indeed, possess their own efficacy in the absence of orthosteric ligand (Christopoulos and Kenakin, 2002; Conn et al., 2009a). This phenomenon presents a unique opportunity to exploit GPCRs as drug targets through offering novel and often less-conserved ligand binding sites across receptor subtypes.

Despite the best-characterised coupling partners of GPCRs being heterotrimeric G proteins, they are also well known to couple to a host of other intracellular proteins (e.g. arrestins and small G proteins (Burridge and Wennerberg, 2004; Lefkowitz, 1998)), thus adding an extra degree of complexity to the pluri-dimensional response of ligand-GPCR interactions. Furthermore, promiscuous coupling has been shown, in some cases, to be a

concentration- and/or oligomerisation-dependent event (Sato et al., 2007; Scholten et al., 2011; Urizar et al., 2011).

Taken together, the ligand-receptor-effector combinations, receptor oligomerisation and allosteric modulation of GPCRs furnish a mode of fine-tuning functional outputs and potentially, therefore, clinical outcomes.

This chapter will focus on two major receptor types of Class C GPCRs, the metabotropic glutamate and metabotropic γ -amino-butyric acid (GABA) receptors, which are the GPCRs of the major excitatory and inhibitory neurotransmitters in the adult brain, respectively. These receptors represent major targets for many CNS disorders such as schizophrenia, Parkinson's disease, Alzheimer's disease, epilepsy and diseases of addiction (Conn et al., 2009a; Tyacke et al., 2010).

2. Metabotropic glutamate receptors

2.1 Phylogeny and structure/function of mGlu receptors

Metabotropic glutamate (mGlu) receptors are widely expressed in the CNS and are activated by the excitatory neurotransmitter, glutamate. These receptors play a vital role in the regulation on neuronal excitability and synaptic transmission (Conn and Pin, 1997). Consequently, these receptors are valuable targets for treating neurological disorders such as schizophrenia, Parkinson's disease and neuropathic pain, either by correcting neurological imbalances in non-glutamatergic systems or through treating dysregulation of glutamatergic signalling.

The members of the mGlu receptor family are obligate dimers and long thought of as obligate homodimers, but have recently been demonstrated to selectively form heterodimers amongst other mGluR subtypes in HEK cells (Doumazane et al., 2011). This propensity may be of utility in texturing the glutamatergic response across diverse brain regions. mGlu receptors consist of 8 subtypes that are divided into three subgroups (I-III) based on sequence homology, function and pharmacological profile (Pin and Acher, 2002). Group I mGluRs (mGlu₁ and mGlu₃) are G_{q/11}-coupled thereby signalling through the phospholipase C-IP₃-Ca²⁺ axis; whereas Group II (mGlu₂ and mGlu₃) and Group III (mGlu₄, mGlu₆, mGlu₇ and mGlu₈) signal through inhibitory G proteins (G_{i/o}), which most likely serve as intermediaries between the receptor and ligand-gated ion channels, such as voltage-operated potassium channels (K_v2 channels) and voltage-operated calcium channels (Ca_v2 channels) (Doupnik, 2008; Herlitze et al., 1996; Peleg et al., 2002).

In drug discovery the understanding of the molecular mechanisms of ligand binding and receptor activation are paramount in order to investigate novel and improved methods for targeting these receptors therapeutically. In this regard, it is important to determine the overall receptor activation event by breaking it down into its fundamental component. Furthermore, to gather information about mGlu receptors, we must also use information gained from studies of other Class C GPCRs to form a global conformational image. Ligand binding in a VFT structure has been described with the periplasmic binding protein, which appears to be similar in class C receptors (O'Hara et al., 1993). The VFT remains in a state of equilibrium between two main conformations: open (o) and closed (c), known as the resting state. The orthosteric ligands bind primarily to the open VFT in lobe 1 and subsequently

promote the closed conformation as interactions with lobe 2 stabilises this state. This suggests that, if agonists induce the closure of the VFT, orthosteric antagonists act to prevent the closure of the VFT, thereby blocking the appropriate mechanisms leading to 7TM activation (Bessis et al., 2000; Bessis et al., 2002; Kunishima et al., 2000; Tsuchiya et al., 2002). For a number of years, the question on how ligand binding in the VFT results in 7TM activation remained to be elucidated. The breakthrough came from the first crystal structures of a class C VFT dimer, from the mGlu1 receptor, crystallised in the presence and absence of glutamate (Kunishima et al., 2000). These structures confirmed the overall structure of the domain and, perhaps more importantly, the agonist binding mode in a single VFT domain. It also revealed large, structural rearrangements of the VFT dimer resulting in a change of the relative orientation of the two protomers. A general mechanism for VFT dimer conformational changes was proposed by the authors: two orientations of the VFT dimer exist and are in equilibrium: a resting (R) and an active (A) orientation. In the R orientation, the VFTs interact via lobe-I only, leaving the lobes-II separate from each other. In the A orientation, there is a reorganization of the VFTs relative orientation such that they also interact via each lobe-II. This large reorientation from R to A was proposed to induce the conformational changes required for 7TM activation. Resting and active designations were given to the different orientations as glutamate was proposed to stabilize the A form. The active and inactive property of the A and R orientations are further supported by mGlu1 structures obtained in the presence of an antagonist (MCPG) or in the presence of a potentiator (Gd^{3+}) in which the dimer orientation is R and A, respectively (Tsuchiya et al., 2002).

When considering the various conformations for the VFT and the VFT dimer, there are a total of six theoretical conformations that are possible: Roo, Rco and Rcc and Aoo, Aco and Acc, where A and R are indicative for the VFT dimer orientation and c and o for the VFT conformation. It is assumed that agonist binding to at least one of the VFT stabilizes the c form, which is the driving force leading to the VFT dimer reorientation from R to A. In agreement, only Roo, Rco, Aco and Acc are likely to exist. However, new crystal structures of the isolated VFT dimer in the 'forbidden' conformation Rcc (Muto et al., 2007) and Aoo (PDB accession number, 3KS9) were recently deposited in the protein data bank (PDB). In particular, the Aoo conformation appears to be highly unlikely to occur within a dynamic equilibrium as many residues of the same polarity from lobe 2 would be in close proximity to one another, so much so that this would likely destabilise this conformation through the repulsive forces exerted within lobe 2 (Tsuchiya et al., 2002). Whilst explanations for these surprising observations have not been provided, the absence of 7TM may have alleviated some conformational constraints that may otherwise be exerted on the VFT from the 7TM, acting as a structural tether that inhibits certain conformations.

A question arising upon closer analysis of the crystal structure is the number of agonists needed to activate a class C GPCR dimer. When considering the reorientation of the VFT from R to A as the sole mechanism responsible for 7TM activation, one may wonder whether there is a functional difference between Aco and Acc conformation. In other words, what would be the difference in binding one or two agonists? It was shown that in class C heterodimers a single subunit was responsible in binding the endogenous ligand (GABAB1 in GABAB receptor and T1R1 or T1R2 in the taste receptors)(Kniazeff et al., 2002; Nelson et al., 2001). This suggests that a single agonist molecule is sufficient to fully activate heterodimeric receptors in these cases.

As we have described above, an allosteric modulator binding in the 7TM affects both the G protein activation and agonist affinity for the VFT. Together with the fact that a conformational change in the VFT dimer activates the 7TM, this indicates that VFT and 7TM converse in both ways. The question that remains is how the stimulus is transduced through the VFT region to the 7TM domain?

In most of class C GPCRs, VFT and 7TM are connected with the CRD. The CRD is an 80 residues long domain containing 9 cysteines. This domain is present in mGlu, CaS, GPRC6A and T1R receptors, but not in GABA_B receptors. The structure of this domain has been solved for mGlu₃ (Muto et al., 2007), and this domain appears to be a rigid 40Å long structure, which is most likely to form a physical gearing system between the VFT and 7TM domains. In agreement with these physical findings, both deletion of the CRD in mGlu or CaS receptors and mutations of T1R3 CRD abolish the agonist-induced receptor activation (Hu et al., 2000; Jiang et al., 2004). Furthermore, we have shown that the VFT and CRD domains in mGlu₂ are linked by a disulphide bridge between a cysteine at the bottom of the VFT and the only cysteine that is not engaged in intradomain disulphide bond within the CRD (Rondard et al., 2006). Rondard et al., had shown that the mutation of the residues involved in this interaction abolished agonist-mediated activation of the receptor. This supports the idea of a central role for the CRD in the transduction of the conformational changes from the VFT dimer to the 7TM in these receptors.

The exact mechanisms of 7TM activation Class C and indeed, mGlu receptors remain to be solved. This notwithstanding, there are approaches that can be employed in an attempt to determine the molecular mechanisms involved in the conformational changes that the 7TM domains undergoes upon activation. One of these approaches is entails the use of both positive and negative allosteric modulators. The first allosteric modulators of class C GPCRs to be described were found to be non-competitive antagonists or inverse agonists (Carroll et al., 2001; Litschig et al., 1999; Pagano et al., 2000). Other compounds have been described that potentiated the effect of the agonists (increased affinity and efficacy) (Felts et al., 2010; Hammond et al., 2010; Urwyler et al., 2001). These molecules are structurally distinct from the orthosteric agonists and antagonists, as reflected in their binding within the 7TM, in a binding region that is reminiscent of the orthosteric binding pocket in Class A receptors (Brauner-Osborne et al., 2007; Goudet et al., 2004). So far, no endogenous PAM or NAM binding in the 7TM pocket has been described. Selective pressure in the evolution of a site/pocket is often indicative of a biological function, but there is no conserved pocket located within the 7TM domain of mGluRs, making it less likely that there is an endogenous allosteric ligand that acts in that region. The absence of conservation allowed the discovery of molecules specific for a single subtype of mGlu receptor, as opposed to a ligand acting at the well conserved orthosteric binding site. If both PAM and NAM act at the 7TM, then their opposite effects are likely due to differences in the residues that the ligands are in contact with in the 7TM. Specifically, several studies indicate that PAM and NAM bind to overlapping but not identical sites (Miedlich et al., 2004; Petrel et al., 2004). Some of these interaction networks should stabilize the active conformation of the 7TM, whilst some others should lock the receptor in its inactive conformation. However, it was shown that structurally different molecules bind essentially at the same position in the 7TM, only the precise identity of the residues contacting the molecule may differ. It appears that the position of PAM/NAM binding site is largely conserved in the whole family and includes residues from TM3, 5, 6 and 7 (Hu et al., 2000; Miedlich et al., 2004; Pagano et al., 2000). However, in some cases, two distinct sites have been

identified for PAMs, as exemplified at mGlu₅ (Chen et al., 2008). See Figure 2 for a schematic overview of mGlu receptor architecture and binding domains.

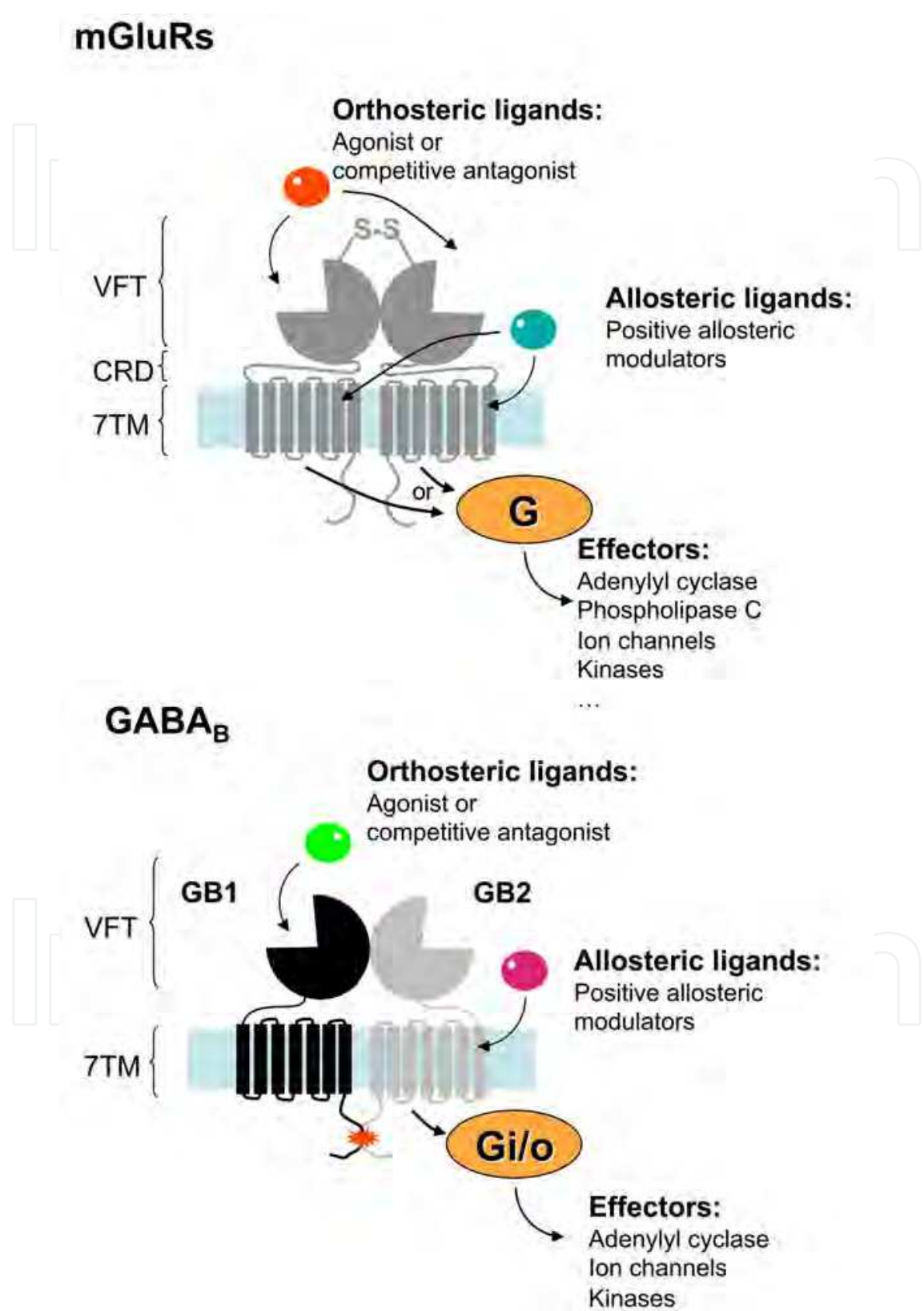


Fig. 2. Architecture, binding domains and dimerisation states of mGlu and GABA_B receptors.

2.2 Protein-protein interactions of mGlu receptors

Studying molecular mechanisms and pharmacology of GPCRs in heterologous cells systems can be exceptionally useful due to the eradication of confounding factors such as multiple receptor subtypes; in addition the capacity to modulate receptor expression and function of specific signalling pathways with relative ease. However, these systems are rarely indicative of native systems and it needs to be recognised that various GPCR interactions exist *in vivo* that do not exist in heterologous cell systems for a myriad of reasons. One such interaction is that of protein-protein interactions, whereby the physical or functional interaction of a number of proteins can greatly alter its behaviour. An example of this occurrence is a fundamental component of some Class C GPCR pharmacology, such that receptor activity-modifying proteins (RAMPs) modulate the pharmacology of receptors such as the calcitonin and calcitonin receptor-like receptor (Sexton et al., 2006). mGlu receptors are also a family that are capable of interacting with non-mGluR proteins to form complexes.

2.2.1 mGlu₁–A₁ receptors

In cortical neurons, the simultaneous activation of adenosine A₁ and mGlu₁ receptors has been shown to synergistically decrease the neuronal toxicity due to application of NMDA (Ciruela et al., 2001). In astrocytes or in co-transfected HEK293 cells, activation of A₁ receptors elicits an increased mGlu₁ response via G_{i/o} proteins (Ciruela et al., 2001; Toms and Roberts, 1999). That effect could be indicative of cross-talk and priming of the intracellular Ca²⁺ response; however, Hirono et al. (2001) did not observe any potentiation of the mGlu₁ response upon co-activation of the A₁ receptor in cerebellar Purkinje cells, supporting the hypothesis of cooperativity (physical or otherwise) rather than cross-talk of the signalling pathways. Although both receptors are co-localized and coimmunoprecipitated from neurons and transfected HEK293 cells, the existence and the requirement of a direct physical interaction is yet to be clearly established (Ciruela et al., 2001).

2.2.2 mGlu₅–A_{2A}–D₂ receptors

The mGlu₅, adenosine A_{2A} and dopamine D₂ receptors are highly expressed in the striatum. These receptors have been proposed to play vital roles in the dysregulation of the motor coordination observed in the Parkinson's disease. Indeed, antagonists of both mGlu₅ and A_{2A} display anti-parkinsonian effects, while the dopamine D₂ receptor is the target of L-DOPA, which is used to treat parkinsonian symptoms. It has been suggested that these three receptors may act in concert in pairs or as a triplet via signalling cross-talk or otherwise, to influence the striatal function in motor coordination (Agnati et al., 2003; Cabello et al., 2009). Indeed, this cross-regulation was observed *in vivo*, where mGlu₅ antagonist-induced motor effects were augmented by A_{2A} receptor antagonists; and conversely these effects were diminished in A_{2A}-D₂ receptor double knock-out mice (Kachroo et al., 2005). The exact molecular mechanisms of the cross-regulation are not well understood, but DARPP-32 (dopamine- and cAMP-regulated neuronal phosphoprotein) may play a pivotal role. Adenosine A_{2A} receptors have been shown to increase DARPP-32 phosphorylation via the G_s signaling axis, whilst D₂ receptors counteract this effect via the G_{i/o} pathway (Agnati et al., 2003); Furthermore, the co-activation of adenosine A_{2A} and dopamine D₂ receptors synergistically potentiated DARPP-32 phosphorylation *ex vivo* studies in striatum tissues. Notwithstanding, the regulation of intracellular Ca²⁺ and cAMP signals underpins other

signalling interactions between these receptors (Ferre et al., 2002). Not only may this phenomenon be due to signalling cross talk amongst these receptors, but may be a result of physical interactions and allosteric regulation across heteromers. It A_{2A} - D_2 hetero-oligomers are mediated by electrostatic interactions between a basic-rich motif in the third intracellular loop of the D_2 receptor and an acidic/serine residue-containing motif in the C-terminus of the adenosine A_{2A} receptor (Azdad et al., 2009; Ciruela et al., 2004; Ferre et al., 2007). Additionally, are postulated to not only be co-expressed, but also to form hetero-oligomers in striatal neurons and in heterologous cells systems (Ferre et al., 2002). Recently, Cabello et al. (2009) demonstrated that mGlu₅, dopamine D_2 and adenosines A_{2A} receptors are localised within the same dendritic spines in glutamatergic striatal synapses, which led them to hypothesise that there may be hetero-oligomeric triplets of A_{2A} , mGlu₅ and D_2 receptors; this association was then investigated through the employment of various fluorescence techniques. Their data supported the formation of heterooligomers containing all three receptors and thus allosterically interacting with one another to influence either efficacy or affinity or both. It is noteworthy that additional cross-regulation between A_{2A} and mGlu₅ receptors has been reported in hippocampal neurons, where the inhibition of A_{2A} receptors decreased the mGlu₅-mediated potentiation of NMDA receptor responses (Tebano et al., 2006). However, the molecular mechanisms involved are yet to be elucidated.

2.2.3 mGlu₂-5-HT_{2A} receptors

One of the best-characterized receptor complex involving a Class C GPCR is the complex between mGlu₂ and the serotonin 5-HT_{2A} receptor. It is well documented that these receptors are both targeted by antipsychotic drugs such as 5-HT_{2A} receptor inverse agonists and mGlu₂ receptor agonists and PAMs (Benneyworth et al., 2008; Benneyworth et al., 2007). Furthermore, 5HT_{2A} receptors are the target of hallucinogenic substances, for example LSD and psilocybin, which induce hallucinogenic episodes that are thought to be similar to some of the symptoms in schizophrenics (Aghajanian and Marek, 1999). Indeed, non-hallucinogenic 5HT_{2A} agonists (5-HT included) activate the G_q signalling axis, whilst hallucinogenic compounds are proposed to additionally activate G_{i/o} and Src tyrosine kinase pathways, in cortical neurons (Gonzalez-Maeso et al., 2007; Gonzalez-Maeso et al., 2003). Activation of mGlu₂ receptors in the prefrontal cortex by the mGlu₂ PAM, biphenyl-indanone A (BINA), abrogated the hallucinogenic effects of compounds such as (-)-2,5-dimethoxy-4-bromoamphetamine, [(-)DOB] (Benneyworth et al., 2007); suggesting functional antagonism between mGlu₂ and 5HT_{2A} receptors in prefrontal cortex, an interaction that is possibly altered in schizophrenics (Gonzalez-Maeso et al., 2007). In fact, co-expression of both receptors revealed that the hallucinogen-induced G_i coupling of 5-HT_{2A} is ameliorated by mGlu₂ in basal conditions, but abolished when mGlu₂ is activated. The mechanism of this complex cross-talk remains to be fully unraveled, but it has been proposed to be the result of mGlu₂-5-HT_{2A} receptor oligomerisation. In cortical neurons, these receptors co-localise and co-immunoprecipitate (Gonzalez-Maeso et al., 2008). Indeed, biophysical approaches have been employed to demonstrate that these GPCRs are in fact in close enough proximity to be compatible with a physical association (Gonzalez-Maeso et al., 2008). Moreover, by adopting a chimeric approach between mGlu₂ and mGlu₃ (TM4 and TM5 substitution), the authors were able to demonstrate that mGlu₃ receptors with substituted TM domains were able to oligomerise with the 5-HT_{2A} receptor, further to exhibiting functional cross-talk (Gonzalez-Maeso et al., 2008). This supports the potential

relationship between receptor oligomerisation and functional cross-talk. The study of the precise mechanism of this phenomenon is still ongoing, and can perhaps furnish novel approaches for targeting these receptors for the treatment of schizophrenia and other neuronal disorders.

2.2.4 mGlu₅-NMDA receptors

Another important interaction that further implicates the role of the glutamatergic system in schizophrenia is the interaction of the *N*-methyl-D-aspartate (NMDA) receptor and mGlu₅. This GPCR-ion channel interaction has been relatively well characterised from a functional stand point, but the molecular mechanisms of the interaction are only beginning to be unfolded.

Indeed, in hippocampal neurons, mGlu_{5a} co-localises with NMDA receptors, which mediates a slow excitatory postsynaptic current (Collingridge et al., 1983; Oliet et al., 1997). The activation of mGlu₅ receptors enhances the NMDA-evoked responses in different regions of the brain, such as the hippocampus, the striatum, the cortex, or the spinal cord (Aniksztejn et al., 1992; Harvey and Collingridge, 1993). Recently, Perroy et al., (2008) have shown that both receptors, indeed, interact via the C-terminal domain of mGlu_{5a}. Through use of the bioluminescence resonance energy transfer (BRET) approach, they demonstrated that a significant and specific BRET signal can be measured between the two receptors, and moreover that this signal was transiently increased by activation of either the mGlu_{5a} receptor or the NMDA receptor; this suggests an allosteric interaction and ligand-dependent conformational rearrangement of the opposite protomer in the hetero-oligomer. Interestingly however, when co-expressed, the functional response of the either receptor was reduced, compared to the response when either receptor was expressed in isolation. Thus suggesting a reciprocal and constitutive suppression of the signalling between NMDA and mGlu_{5a} receptors, which was suggested to be independent of the G protein coupling of mGlu_{5a}. The inhibitory reciprocal effect was dependent on the physical interaction between these receptors, given that the inhibition was abolished upon suppression of the C-terminal domain involved in receptor hetero-oligomerisation (Perroy et al., 2008).

2.3 Localisation and physiological function

Group I mGlu receptors (mGlu₁ and mGlu₅) are extensively expressed throughout neurons in the CNS and, in addition, mGlu₅ is expressed in glial cells. mGlu₁ is most abundantly expressed in Purkinje cells of the cerebellar cortex and in the olfactory bulb, in addition to strong expression in the hippocampus, substantia nigra and globus pallidus (Baude et al., 1993; Martin et al., 1992); and mGlu₅ is greatly expressed in corticolimbic regions, such as the striatum, hippocampus and cerebral cortex (Ferraguti and Shigemoto, 2006). For example in the hippocampus, mGlu₁ has been demonstrated to be involved in synaptic transmission and plasticity, in addition to neuronal excitability (Bortolotto et al., 1999), whilst in both mGlu₁ and mGlu₅ are required for the induction of long-term depression (LTD) in corticostriatal synapses (Sung et al., 2001). Through the use of knockout (KO) mice the putative function of mGluRs can be elucidated and, indeed, mGlu₁ and mGlu₅ KO mice have been studied. In mGlu₁ KO animals is a marked deficits in long-term potentiation (LTP) in hippocampal slices and in context-dependent fear conditioning task (Aiba et al., 1994a); suggesting reduced hippocampal-mediated learning and memory. Furthermore,

these mice are also cerebellar-LTD deficient, suggesting that mGlu₁ receptors are important for LTD induction in the cerebellum and subsequently motor learning, as demonstrated by the ataxic gait of the mGlu₁ KO mice (Aiba et al., 1994b). Recently, mice have been generated whereby the mGlu₅ gene can be selectively disrupted in the central nucleus of the amygdala; these mice exhibited a lack of mechanical hypersensitivity induced by peripheral inflammation (Kolber et al., 2010), strongly suggesting a role of mGlu₅ in the regulation of inflammatory pain transmission. Both mGlu₁ and mGlu₅ KO mice exhibit deficiencies in prepulse inhibition of the startle reflex, which is an indicator of sensorimotor gating that is impaired in schizophrenic patients, a trait that can be reversed through treatment with antipsychotics (Brody et al., 2003; Brody et al., 2004).

mGlu₂ and mGlu₃ (Group II) are widely expressed in the CNS, of which mGlu₂ is more limited in expression compared to mGlu₃. mGlu₂ expression has been observed in Golgi cells of the cerebellar cortex and in mitral cells of the accessory olfactory bulb (Ohishi et al., 1998; Ohishi et al., 1994). mGlu₃ receptors have been observed in the olfactory tubercle, neocortex, limbic cortex, and is also present in Golgi cells of the cerebellar cortex (Tamaru et al., 2001). Similar to Group I mGlu receptors, KO mice have also been generated for Group II mGluRs, with both mGlu₂ and mGlu₃ KO mice exhibiting a loss of mGlu_{2/3} agonist, LY354740-induced anxiolytic behaviour in an elevated plus maze test (Linden et al., 2005). Further to this, mGlu₂, but not mGlu₃ KO mice displayed a loss of Group II agonist-mediated antipsychotic behaviour (Fell et al., 2008; Woolley et al., 2008), highlighting the role of mGlu₂ in anxiety and psychotic behaviours. Interestingly, in addition to these functions, Group II mGlu receptors have also been demonstrated to modulate the release of other neurotransmitters, for example, LY354740 reduced KCl-induced [³H]-GABA release in rat primary cortical cultures, this effect was then reversed with the mGlu_{2/3} antagonist, LY341495 (Schaffhauser et al., 1998).

Group III mGluRs (consisting of mGlu₄, mGlu₆, mGlu₇ and mGlu₈) are mainly expressed on presynaptic neurons throughout the CNS, with the exception of mGlu₆, which is expressed postsynaptically on retinal ON bipolar cells (Nakajima et al., 1993). mGlu₄ is highly expressed in the cerebellum and consequently, mGlu₄ KO mice experience deficits in spatial memory (Gerlai et al., 1998) and learning of complex motor tasks (Pekhletski et al., 1996). mGlu₆ KO display deficits in ON response to light stimulation, yet the OFF response remained unchanged (Masu et al., 1995), highlighting the importance of mGlu₆ in synaptic neurotransmission in retinal ON bipolar cells. mGlu₇ deficient mice display learning and memory deficits, in addition to exhibiting an epileptic phenotype (Bushell et al., 2002; Sansig et al., 2001). Both mGlu₇ and mGlu₈ KO animals display increase anxiety (Cryan et al., 2003; Duvoisin et al., 2005).

As previously mentioned, the mGluR family of receptors are expressed widely through the CNS and exhibit a wide number of functions; moreover through KO studies, we can deduce the key roles played by each mGluR subtype and subsequently tailor our pharmacological armamentarium accordingly.

2.4 Pharmacology and clinical relevance

2.4.1 Ligands for group I mGlu receptors

The first selective orthosteric agonist at mGlu₁ and mGlu₅ receptors is (S)-3,5-dihydroxyphenylglycine, [(S)-3,5-DHPG], and this remains the case given that ligands such

as quisqualate and [(1*S*,3*R*)-ACPD] also bind to ionotropic glutamate and other mGluR subtypes, respectively (Niswender and Conn, 2010). A range of other orthosteric ligands have been generated, but have limited use due to their low affinity and/or potency. As previously discussed, mGlu receptor subtype selectivity is difficult to obtain due to the high degree of sequence and structural homology between subtypes.

Therefore, one approach is to target non-canonical ligand-binding sites; from this strategy a major breakthrough in Group I mGlu receptor pharmacology was made, with the discovery of CPCCOEt, which was the first mGlu₁ negative allosteric modulator (NAM) (Annoura et al., 1996). CPCCOEt was later discovered to bind to an allosteric domain and this highlighted the capacity of ligands to bind in allosteric binding modes, thereby modulating orthosteric ligand function (Litschig et al., 1999). Thereafter, structurally distinct NAMs for mGlu₁ were also discovered such as BAY36-7620 and FTIDC (Carroll et al., 2001; Suzuki et al., 2007). mGlu₅ selective NAMs were also identified of which the two flagship molecules were MPEP and MTEP, both providing good potency and selectivity (Anderson et al., 2002; Gasparini et al., 1999).

In addition to NAMs, a wide variety of PAMs have also been identified and characterised. Two of these PAMs, Ro 67-4853 and Ro 01-6128 both potentiated DHPG-mediated VOCC inhibition responses in CA3 neurons, but did not exhibit any agonist activity of their own, suggesting their main characteristic is the allosteric potentiation of orthosteric ligand binding and/or efficacy (Knoflach et al., 2001). Interestingly, these PAMs were found to bind to a topographically distinct domain to the NAM binding region, when they failed to displace the well-characterised allosteric antagonist, R214127 (Hemstapat et al., 2006). These data suggest that mGlu₁ possesses multiple allosteric binding sites, in addition to its orthosteric ligand-binding site. Similar to mGlu₁, mGlu₅ PAMs have also been discovered, such as DFB, CPPHA, CDPPB, VU29, and ADX47273, with CDPPB also having some PAM activity at mGlu₁ (Conn et al., 2009b; Hemstapat et al., 2006).

2.4.2 mGlu₁ in anxiety and depression

Anxiety and depression are two of the most common mental disorders, with a lifetime prevalence of approximately 17% and 12%, respectively (Andrade et al., 2003; Depping et al., 2010). It has now been well documented that mGlu₁ receptors and the glutamatergic system represent tractable targets for treating these common disorders (Bittencourt et al., 2004; Paul and Skolnick, 2003).

Anxiety results from an imbalance between GABAergic and glutamatergic systems, either from overactive glutamatergic neurotransmission or inadequate GABAergic activity in hypothalamus, periaqueductal gray, hippocampus and prefrontal cortex (Engin and Treit, 2008). It is hypothesised that the antagonism of mGlu₁ receptors is capable of augmenting the GABAergic response, whilst concomitantly decreasing the NMDA receptor-mediated glutamatergic response in key brain regions involved in anxiety. It has been demonstrated that intraperitoneal administration of the mGlu₁ antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA), rats exhibited anxiolytic-like behaviours in the conflict drinking test and in elevated plus maze tests (Klodzinska et al., 2004). This reinforces the results seen by Chojnacka-Wojcik et al., (1997) where intrahippocampal injection of the Group I mGlu receptor antagonist, (S)-4-carboxy-3-hydroxyphenyl-glycine (S-4C3H-PG), reduced anxiety-

like behaviours in rats. The anxiolytic actions of mGlu₁ blockade were further confirmed through the study of the mGlu₁-selective antagonist, JNJ16259685 (Steckler et al., 2005). This study demonstrated that treatment with JNJ16259685 alleviated the suppression of the licking response in a conflict drinking test, which is consistent with other well characterized anxiolytic drugs (Petersen and Lassen, 1981). However, JNJ16259685 treatment did not induce anxiolytic-type behaviour in elevated plus maze tests, the authors thus postulating that the effects of JNJ16259685 be context specific (Steckler et al., 2005).

Depression is a complex disorder involving the interplay between different neurotransmitters, including noradrenaline, serotonin, dopamine and glutamate (Paul and Skolnick, 2003). Drugs for the treatment for depression are generally based on increasing the lifetime of biogenic amines, such as noradrenaline and serotonin, in the synaptic cleft, for example fluoxetine and escitalopram, which are inhibitors of serotonin- and serotonin and noradrenaline-reuptake transporters, respectively. Over the past decade, it has become more recognised that the glutamatergic system may also play a vital role in the regulation of depression, specifically NMDA receptors, where NMDA receptor expression was reduced in post-mortem depressive brains (Feyissa et al., 2009). This theory was retrospectively reinforced by evidence that NMDA receptor antagonists produce anti-depressant effects, whereby competitive and non-competitive antagonists of NMDA receptors, 2-amino-7-phosphonoheptanoic acid (AP-7) and Dizolcipine (MK-801) emulated anti-depressant effects of gold standard anti-depressants (Trullas and Skolnick, 1990). Given the regulatory link between mGlu₁ and NMDA receptors it was postulated that mGlu₁ receptor antagonists or NAMs could mimic the anti-depressant effect of NMDA receptor inhibitors. The mGlu₁ antagonist, JNJ-16567083 has been shown to be efficacious in despair-based animal models of depression, specifically forced swim test and tail suspension test (Belozertseva et al., 2007; Molina-Hernandez et al., 2008).

2.4.3 mGlu₅ and schizophrenia

Schizophrenia is a complex multi-faceted disease that manifests itself as a host of symptoms such as paranoia, social withdrawal and delusions, along with a number of cognitive deficits. Given that there is no single causative factor, there is some difficulty in finding a suitable target. Current first-line treatment involves broad-spectrum biogenic amine (e.g. dopamine, serotonin, acetylcholine) receptor antagonists, but these do not satisfactorily treat the cognitive symptoms. The underlying rationale of this approach is to decrease dopaminergic neurotransmission in thalamocortical and limbic circuits. One potential mode of treating schizophrenia lies within targeting GABAergic and glutamatergic interneurons in pivotal cortical and limbic regions, specifically, the dysregulation of the disinhibition of glutamatergic neurotransmission (Chavez-Noriega et al., 2002; Coyle, 2006). The blockade of N-methyl-D-aspartate (NMDA) receptors on these interneurons results in a glutamatergic disinhibition, which in turn leads to an overexcitability of thalamocortical neurons, which is mostly mediated by DL-a-amino-3-hydroxy-5-methylisoxasole-4-propionate (AMPA) receptors in thalamocortical synapses. Within these regions NMDA and mGlu₅ receptors have been demonstrated to functionally and physically interact, i.e. the activation of mGlu₅ receptors increases the activity of NMDA receptors on GABAergic and glutamatergic neurons (Conn et al., 2009b); it is thus postulated that the activation of mGlu₅ can be employed as a means to decrease neuronal excitability in thalamocortical regions. This hypothesis is reinforced through knockout studies, whereby the knockout of mGlu₅ resulted

in NMDA-dependent cognitive and learning deficits (Lu et al., 1997). Therefore, adopting an mGlu₅ agonist or PAM could alleviate the cognitive symptoms in schizophrenic patients; moreover, the use of a PAM will allow relatively specific mGlu₅ in the afflicted region whilst maintaining the spatio-temporal regulation of other mGlu₅-containing neurons. Indeed, the abovementioned mGlu₅ PAM, CDPBB, which has a suitable potency and solubility profile for *in vivo* studies, has been demonstrated to decrease amphetamine-induced disruption of prepulse inhibition (PPI) startle response and locomotor activity (Kinney et al., 2005); and to increase hippocampal synaptic plasticity, an important feature in cognition (Ayala et al., 2008; Conn et al., 2009b).

2.4.4 Group II mGlu receptor pharmacology

Group II mGlu receptors (mGlu₂ and mGlu₃) are generally localised presynaptically and negatively regulate cAMP signalling, and moreover, VOCCs. As with nearly all orthosteric mGlu pharmacological agents there is the underlying issue of selectivity. DCG-IV and LY379268 are reference Group II mGlu agonists, BINA and LY487379 are highly potent PAMs and the recently discovered MNI series of compounds (MNI-135, MNI-136 and MNI-137) are potent negative allosteric modulators (Galici et al., 2006; Hemstapat et al., 2006; Johnson et al., 2003; Linden et al., 2005; Schweitzer et al., 2000). Despite the large array of pharmacological tools available for Group II mGlu receptors, there remains a paucity of ligands that selectively differentiate between mGlu₂ and mGlu₃, which is due to the high degree of sequence homology between the two. Of lesser therapeutic relevance, there are also Group II mGlu receptor antagonists, such as 2S-2-amino-2-(1S,2S-2-carboxycyclopropan-1-yl)-3-(xanth-9-yl)propionic acid (LY341495) and (1R,2R,3R,5R,6R)-2-amino-3-(3, 4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0] hexane-2,6-dicarboxylic acid (MGS0039), which have been suggested to have some anti-depressant and anti-obsessive-compulsive characteristics; however they are mostly used as pharmacological tools (Palucha and Pilc, 2005; Shimazaki et al., 2004). Given the lack of selectivity across Group II mGlu receptors it is difficult to pharmacologically distinguish the roles of each receptor in various animal models of disease states without the use of knockout animals.

2.4.5 Group II mGlu receptors in addiction

Addiction is a unique disorder in that it is not only a physiological dependence, but is also a psychological dependence on, canonically, drugs of abuse. It is believed that mGlu₂/mGlu₃ receptor ligands could be capable of treating addiction to such substances as cocaine and nicotine. In fact, not only is it that mGlu₂/mGlu₃ receptor activation is involved in recovery of a dysfunctional system in the corticolimbic system, but it has been shown that the function of Group II mGlu receptors is impaired, either by receptor downregulation or dampening of the G protein-mediated signalling, after acute and chronic stimulation by nicotine, cocaine and ethanol (Bowers et al., 2004; Kenny and Markou, 2004; Neugebauer et al., 2000). Indeed, mechanistically, the decrease in function is hypothesised to be due to an alteration in expression of the activator of G protein signalling 3 (AGS3), whereby AGS3 is overexpressed during withdrawal of repeated dosing of cocaine (Bowers et al., 2004). The authors went on to postulate that AGS3 gates expression of cocaine-induced plasticity in prefrontal cortex, via the regulation of G protein signalling. Furthermore, the downregulation of mGlu₂/mGlu₃ receptors has been observed during cocaine withdrawal

periods, specifically these receptors were downregulated in the shell and core of the nucleus accumbens (Ghasemzadeh et al., 2009). These alterations in expression and function in turn results in an impairment of long-term depression (LTD) in nucleus accumbens and prefrontal cortex in response to chronic morphine and cocaine exposure, respectively (Moussawi and Kalivas, 2010); similarly, a reduced activation of mGlu₂/mGlu₃ receptors resulted in a decrease in long-term potentiation (LTP) after self-administered cocaine withdrawal (Moussawi et al., 2009). Indeed, it is well documented that mGlu₂/mGlu₃ function is altered in the case of substance withdrawal, however the system is regulated in a manner of ways. Explicitly, Group II mGlu receptors are involved in the circuitry that leads to reward processing and addictive behaviour. The activation of mGlu₂/mGlu₃ receptors with the orthosteric agonist, LY379268 resulted in the attenuation of the reinstatement of cocaine-seeking behaviour after exposure, compared to a conventional reinforcer (in this case, sweetened condensed milk) (Baptista et al., 2004). The authors proposed that this was a cocaine-specific effect and was most likely related to the mechanism of action of cocaine itself. Functionally, this regulation may lie in the pre-activation of mGlu₂ receptors, whereby in mGlu₂ knockout mice there was an increased release of glutamate and dopamine in response to cocaine, in the nucleus accumbens (Morishima et al., 2005). Whilst this does provide some evidence on how glutamate is involved in reward circuitry, one must remain circumspect on their conclusions given any compensatory mechanisms are not accounted for.

2.4.6 Group III mGlu receptors and their ligands

For many years, much of the drug discovery efforts have been directed towards Group I and II receptors to exploit their roles in central nervous disorders such as schizophrenia and neuropathic pain. However, of late, efforts have been turned to developing selective ligands for Group III as novel targets for disorders, for example, Parkinson's disease. The prototypical Group III-selective orthosteric agonist is L-amino-4-phosphonobutyrate (L-AP4), yet this ligand is only selective for Group III mGlu receptors, not within the group. In an attempt to ameliorate the affinity and potency, a series of constrained cyclic forms of glutamate were generated and so was created aminocyclopentane-1,3,4-tricarboxylate (ACPT-I), which showed mildly enhanced potency at mGlu₄ and mGlu₈ compared to mGlu₅ and mGlu₆ (Acher et al., 1997; Schann et al., 2006). Similar to the agonists, there are only selective antagonists for Group III mGlu receptors, but not within the group. For example, there are the α -methyl analogues of L-AP4 and L-SOP, specifically MAP4 and MSOP, respectively, with affinity in the micromolar range (Wright et al., 2000). In addition to these, there are the hallmark antagonists of mGlu receptors such as DCG-IV and LY341495, which both have reasonable affinity for Group III mGlu receptors, but also have strong affinity at Group I and Group II receptors, respectively; notably, DCG-IV is also a Group II mGlu receptor agonist (Brabet et al., 1998). Allosteric modulators that act in the 7TM domain Group III mGlu receptors have also been characterised, specifically N-Phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1acarboxamide (PHCCC) and cis-2-([(3,5-Dichlorophenyl)amino]carbonyl)cyclohexanecarboxylic acid (VU0155041), which are both PAMs at mGlu₄ (Niswender et al., 2008); 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-c]pyridine-4(5H)-one hydrochloride (MMPIP), a NAM for mGlu₇ (Niswender et al., 2010); however there remains a relative paucity of allosteric modulators for mGlu₆ and mGlu₈.

One pharmacological avenue that is only beginning to be explored at Class C GPCRs is that of extracellular domain allosteric modulators. For the umami taste receptors, it has been long known that purinergic ribonucleotides, such as inosine- and guanine-monophosphate molecules (IMP and GMP) were potent positive allosteric modulators of the L-glutamate action at the umami receptor (Yamaguchi and Ninomiya, 2000). Interestingly, mutants that altered the effects of glutamate effect were also enhanced by IMP and GMP (Zhang et al., 2008). By employing a chimeric approach along with mutagenesis and molecular modelling, sweet-umami receptors were analysed and the mode of binding and action of IMP was postulated; specifically, the residues lining the IMP binding pocket at the sweet-umami taste receptor, T1R1, were determined (Zhang et al., 2008). It was demonstrated that IMP binds to a novel site that is adjacent to the glutamate binding pocket, the authors thus proposed a model for ligand cooperativity for the mechanism of action of IMP in the T1R1 VFT. The binding of L-glutamate close to the hinge region of the VFT would stabilize the closed conformation of the domain; moreover, binding of 5' ribonucleotides to an adjacent site closer to the putative entrance of the VFT would further stabilize the closed conformation, thereby potentiating the affinity and/or efficacy of L-glutamate. At mGlu receptors, the glutamate-binding pocket is well conserved across the mGlu subtypes, encumbering the discovery selective orthosteric agonists and antagonists (Brauner-Osborne et al., 2007). However, recently, long alkyl chain containing derivatives of (R)-PCEP, a molecule discovered by virtual screening on the VFT of mGlu receptors, revealed a new binding pocket in mGlu₄ (Selvam et al., 2010). Indeed, these compounds not only bind in the glutamate-binding pocket itself, but may also interact with a novel, putative binding pocket adjacent to the glutamate-binding site. Given this new interacting region is formed with residues that are less conserved across the eight mGlu subtypes, this mode of targeting mGlu receptors may furnish compounds with greater selectivity. One such compound may already exist in LSP1-2111, with its L-AP4-like moiety and a 4-hydroxy-3-methoxy-5-nitro-phenyl moiety, it is possible that this molecule bridges across two distinct binding domains, in a similar fashion to bitopic ligands at muscarinic receptors (Antony et al., 2009; Valant et al., 2008; Valant et al., 2009). Accordingly, this ligand has superior selectivity at mGlu₄ and mGlu₆ over mGlu₇ and mGlu₈ (Beurrier et al., 2009).

For an overview of chemical structures of a small range of classical orthosteric mGlu receptor ligands, refer to Figure 3 below.

2.4.7 Group III mGlu receptors and Parkinson's disease

Parkinson's disease is one of the most common of neurological disorders, which is largely characterised by its effects on motor function, such as bradykinesia and dyskinesia; further to other non-motor symptoms, for example pain and gastrointestinal dysfunction. Parkinson's disease arises mostly due to a progressive degeneration of dopaminergic neurons in the substantia nigra, leading to excessive cholinergic neurotransmission in the striatum (Pisani et al., 2003). Subsequently, the inhibitory effect that dopamine provides in these circuits augments GABAergic firing in the striatopallidal pathway leading to excessive inhibition of GABAergic neurons in the subthalamic nucleus, in turn leading to the abnormal enhancement of glutamatergic neurons (Hirsch, 2000). Currently, the frontline treatment is levo-dopa, which compensates for the diminished dopaminergic function. However, the activation of presynaptic mGlu₄ specifically, may result in the diminution of

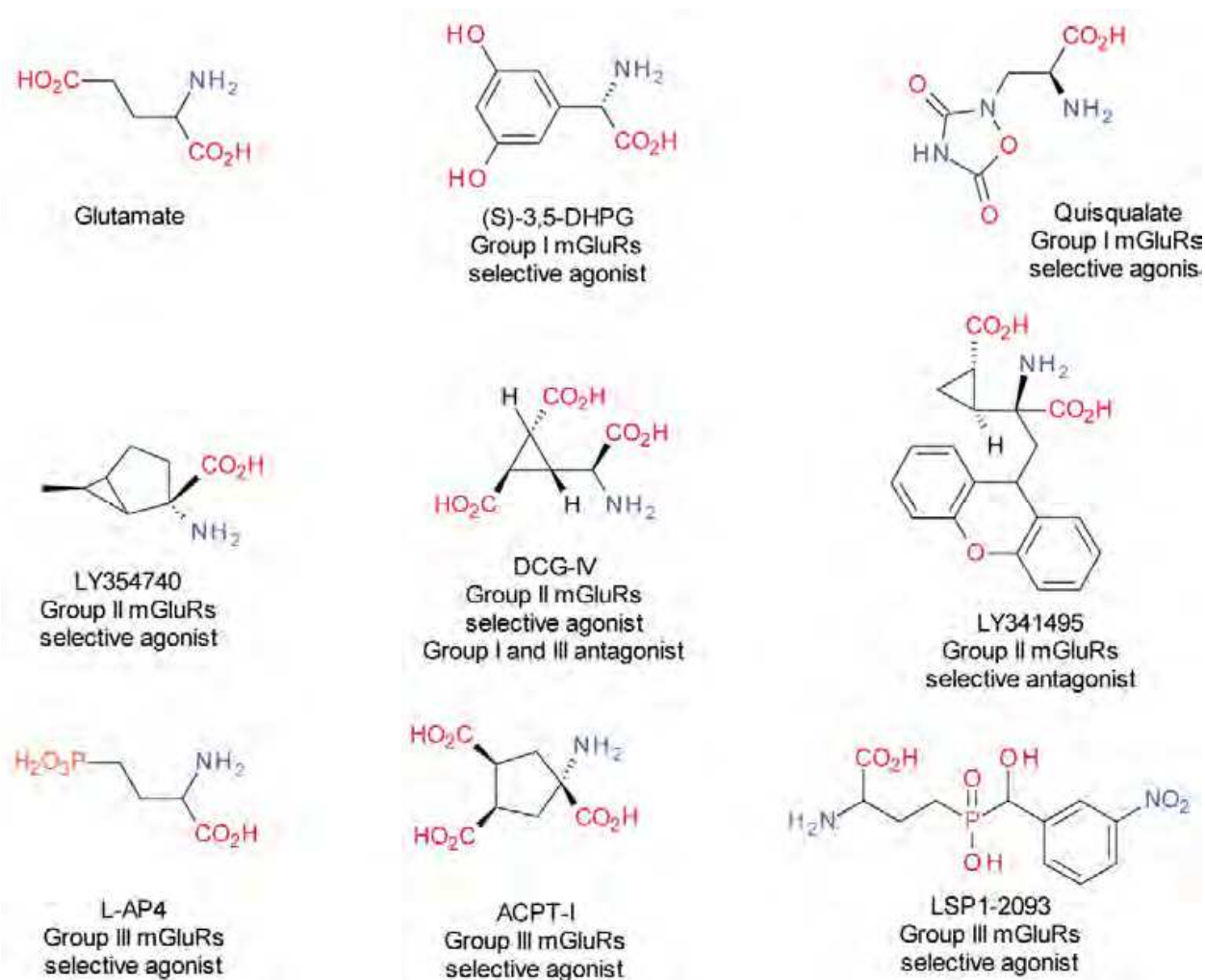


Fig. 3. Highlighting the structural diversity of agonist and antagonists of mGlu receptors.

increased GABAergic firing in striatopallidal projections. Indeed, compounds that have relatively good selectivity for mGlu₄ have been demonstrated to depress the GABA-mediated inhibitory synaptic transmission and relieve motor symptoms in animal models of Parkinson's disease (Beurrier et al., 2009; Valenti et al., 2003). Given that the dopaminergic dysfunction in the substantia nigra and inhibition of GABA signalling by mGlu₄ in the globus pallidus are not inextricably linked there is potential that prolonged mGlu₄ receptor activation will result in less compensatory over-activation of the dopaminergic system, therefore maintaining the therapeutic activity of mGlu₄ targeting ligands (Nicoletti et al., 2011). Indeed, it has been shown that the *in vivo* treatment with the mGlu₄ PAM, PHCCC, reduced dopaminergic neurodegeneration in substantia nigral projections in an MPTP-induced Parkinsonism model (Battaglia et al., 2004; Maj et al., 2003). Along with PHCCC, a more recent PAM of mGlu₄ has been characterised and has demonstrated anti-parkinsonian effects (Niswender et al., 2008). VU0155041 is an allosteric agonist and positive allosteric modulator with potency nearly 10-fold of that of PHCCC, moreover, VU0155041 concentration-dependently diminished haloperidol-induced catalepsy and reversed reserpine-mediated akinesia in mice, with an effect that persisted longer than that of the reference Group III orthosteric agonist, L-AP4 (Niswender et al., 2008).

Despite receiving much of the attention within Group III mGlu receptors, mGlu₄ is not alone in its involvement in Parkinson's disease. There remains the possibility that post-synaptic mGlu₇ and mGlu₈ have some effect on the neuronal circuitry in question. The mGlu₇ allosteric agonist, *N,N'*-dibenzhydryl-ethane-1,2-diamine dihydrochloride (AMN082) may inhibit the release of [³H]-D-aspartate in substantia nigral slices, suggesting that selective targeting of mGlu₇ may yield similar results to those at mGlu₄ (unpublished data; Duty, 2010). Despite there being a large amount of doubt surrounding the therapeutic potential of mGlu₈ for the treatment of Parkinson's disease, where the semi-selective mGlu₈ agonist was failed to reverse haloperidol-induced catalepsy (Lopez et al., 2007); administration of the mixed AMPA antagonist/mGlu₈ agonist, (*R,S*)-3-4-DCPG, decreased amphetamine- but not phencyclidine-induced hyperactivity (Ossowska et al., 2004). Concomitantly, (*R,S*)-3-4-DCPG actually enhanced haloperidol-induced catalepsy and induced catalepsy when administered alone. Taken together, and despite similar expression and function compared to mGlu₄, does not appear to be a good candidate target for the treatment of Parkinson's disease. Indeed, this scenario highlights the inherent difficulties that are encountered in the search for mGlu receptor subtype-selective therapeutics.

Taken together, it seems that the most appropriate and effective methods for targeting mGlu receptors is via their allosteric ligand-binding site, which increases subtype selectivity and does not impede normal neurotransmission. Refer to Figure 4 for the chemical structures of some allosteric ligands for mGlu receptors.

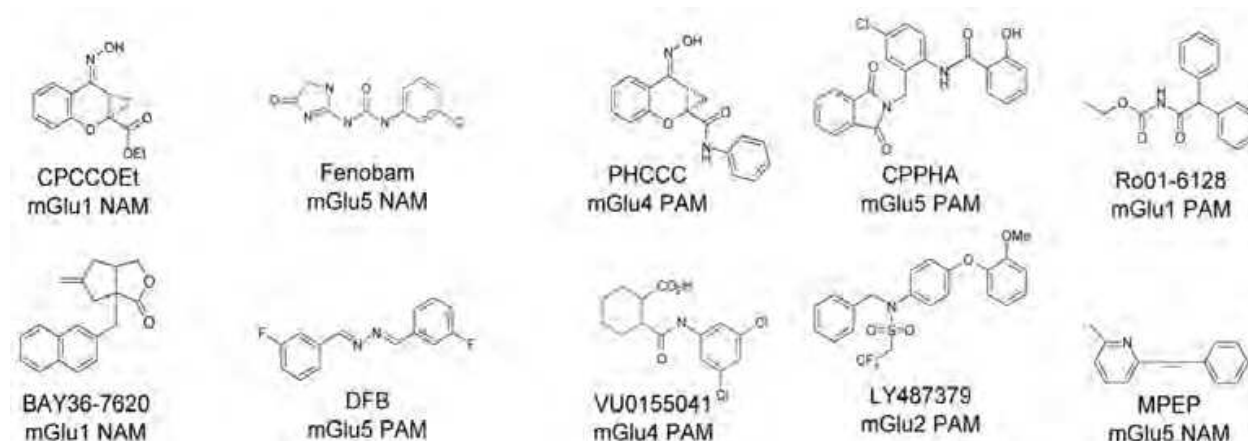


Fig. 4. Chemical structures of mGlu receptor allosteric ligands.

2.4.8 Clinical trials for mGlu receptor ligands

It is now well established that mGlu receptors are major targets for numerous central disorders and even for some in the periphery. Accordingly, there are a large number of clinical programs that are running at any one time (Table 1).

Gastro-(o)esophageal reflux disease (GERD) is a chronic condition, in which the major symptom is the abnormal reflux of stomach contents into the oesophagus. The inhibition of mGlu₅ is predicted to improve the tone of the cardiac sphincter, thus reducing reflux (Lehmann, 2008). In a recent phase II clinical study performed by Addex pharmaceuticals, reflux and other GERD symptoms are efficiently reduced by a NAM of mGlu₅. The same molecule has also entered into a different phase II study targeting migraine, which also

yielded beneficial results. Since glutamate is the main neurotransmitter of the migraine circuit, then inhibition of postsynaptic mGlu₅ receptors that are present in this circuit would decrease glutamatergic neurotransmission and hence may pose a useful approach in migraine therapy. However, due to liver toxicity after long-term treatment with this particular molecule, the study was discontinued. Fragile X syndrome is the most common form of inherited mental retardation. Preclinical studies indicate that fragile X phenotypes are linked to an overactivity of mGlu₅ (Dolen et al., 2010), suggesting that antagonism of this receptor could be of therapeutic interest. Recently, fenobam, an mGlu₅ NAM also known for its anxiolytic properties, entered phase II clinical studies, which so far have demonstrated potential therapeutic benefits on Fragile X symptoms (Berry-Kravis et al., 2009).

Target	Ligand	Company	Trial Phase	Indication
mGlu _{1/5}	Antagonist	Forest Laboratories	Preclinical	Anxiety/Depression
mGlu ₂	TS-032 (Agonist)	Pfizer	Preclinical	Schizophrenia
mGlu _{2/3}	ADX1149 (PAM)	Addex Pharmaceuticals	Phase I	Schizophrenia/ Alzheimer's/ Depression
mGlu _{2/3}	LY2140023 (Agonist)	Eli Lilly	Phase II	Schizophrenia
mGlu _{2/3}	Agonist	Eli Lilly	Phase III*	Anxiety
mGlu ₄	PAM	Merck	Preclinical	Parkinson's disease
mGlu ₅	ADX48621 (Antagonist)	Addex Pharmaceuticals	Phase I	Parkinson's disease
mGlu ₅	AZD2516 (Antagonist)	Astra Zeneca	Phase I	Chronic pain
mGlu ₅	AZD2066 (Antagonist)	Astra Zeneca	Phase II	Chronic pain/GERD
mGlu ₅	NPL-2009	Neuropharm	Phase II	Fragile X syndrome

Table 1. mGlu receptor ligands currently undergoing clinical trials. Sources: ClinicalTrials.gov and EvaluatePharma.com. * - Trial discontinued.

mGlu₂ and mGlu₃ receptors are a major target for the treatment of anxiety and schizophrenia (Conn and Jones, 2009; Conn et al., 2009b). As a result, the activation of these receptors has been exploited for the treatment of said diseases in several clinical studies. Non-selective mGlu₂/mGlu₃ agonists have reached phase II clinical studies for the treatment of generalised anxiety disorders, but the trial was terminated due to risks of seizure observed in animals (Dunayevich et al., 2008). Allosteric ligands represent an alternative to the use of orthosteric ligands, since they do not interfere with the spatiotemporal profile of the endogenous ligand; therefore they are more targeted and usually produce less deleterious side effects. Recently, a phase I study on anxiety was started by Ortho-McNeil-Janssen Pharmaceuticals Inc. and Addex pharmaceuticals using ADX71149, an mGlu₂ PAM, but the conclusions remain known. Altered glutamatergic neurotransmission is also linked in part to schizophrenia and through a phase II study by Eli Lilly, the improvement of

symptoms of schizophrenia with an mGlu₂/mGlu₃ agonist was similar to that demonstrated with olanzapine, a common antipsychotic drug; this drug was also tolerated by patients (Patil et al., 2007).

Preclinical studies strongly suggest that Group III mGlu receptors may play a vital role in the symptomatic control of Parkinson's disease. In particular, increasing mGlu₄ activity within the basal ganglia appears to be an interesting approach to reduce akinetic symptoms associated with Parkinson's disease (Beurrier et al., 2009; Lopez et al., 2007). However, to our knowledge, none of these compounds have reached phase I clinical trials.

3. Metabotropic GABA receptors

3.1 Structure/function of GABA_B receptors

The metabotropic GABA (GABA_B) receptor is the only known GPCR that is responsive to GABA. Architecturally, it is not composed in the same manner as many other Class C GPCRs. Specifically, it consists of a ligand binding GB₁ subunit and a G protein coupling GB₂ subunit (Galvez et al., 2001; Kaupmann et al., 1998; Margeta-Mitrovic et al., 2001; White et al., 1998); each subunit consisting of a VFT and 7TM domains, but converse to mGlu receptors they lack a CRD (refer to Figure 2 for schematic overview). The two subunits are not covalently associated, but do interact via a coiled-coil domain in their C-terminal tails, which provides a solid hydrophobic interaction to maintain the integrity of the dimer (Kammerer et al., 1999). Through the use of circular dichroism spectroscopy the authors proposed a region in the C-terminal domains of GB₁ and GB₂ of approximately 30 amino acids, composed of roughly 5-7 heptads.

Discerning the number of ligands that bind to any one dimer at any one moment is often difficult, especially if there is the possibility for receptors to form higher-order oligomers. It has been shown that in class C heterodimers a single subunit was responsible for the binding of the endogenous ligand, in this case GB₁ in the GABA_B receptor (Kniazeff et al., 2002). This suggests that a single agonist molecule is sufficient to fully activate heterodimeric receptors, but does not discount multiple binding sites on the same protomer. However, nearly nothing is known of the conformational movement of the GB₂ subunit, making it nearly impossible to distinguish between the conformational rearrangement and functional responses of Aco and Acc combinations. The only insights come from the GABA_B receptor, whereby the introduction of several large residues, such as tryptophan in the crevice of GB₂ VFT leads to a decrease in G protein-mediated functional responses (Kniazeff et al., 2002).

It has always been questioned whether GPCRs remain in simple monomeric and dimeric forms or whether they self-associate into higher-order oligomers and, if so, what are the molecular determinants of these interactions. Recently, it has been demonstrated that GABA_B are indeed capable of forming tetrameric complexes, which interact via their GB₁ subunits (Comps-Agrar et al., 2011; Maurel et al., 2008). By employing the use of a binding-null GB₁ subunit Comps-Agrar et al., (2011) demonstrated that GABA_B receptor tetramers could be disrupted and that the resultant complexes are capable of binding approximately twice as much radioligand compared to the wild-type; in addition to increasing the apparent E_{max} in functional tests. The synthesis of this study was that GABA_B receptors that are

associated into a tetrameric assembly have reduced binding capacity and functional capability compared to GABA_B receptors in dimeric form. Comps-Agrar et al., (2011) attempted to more precisely examine the structural determinants of the molecular construction of the GABA_B receptor tetramer. They resolved that an important interaction between the VFTs of the GB₁ subunits occurs, and then experimentally demonstrated the disruption of this interaction through mutation and insertion of an *N*-glycosylation site (G³⁸⁰N) increases the apparent B_{\max} of fluorescent ligand binding and maximal function effect in intracellular calcium mobilisation assays. It is noteworthy that this study demonstrated that there is tetramerisation of GB_{1A} subunit-containing GABA_B receptors, but not GB_{1B} subunit-containing receptors.

Stimulation of GABA_B receptors results in the activation and dissociation of G_{i/o} family G proteins, which in turn inhibit the function adenylyl cyclase thereby decreasing intracellular cAMP levels; activate Kir3 channels and inhibit Ca_v2 channels (Dunlap and Fischbach, 1981; Leaney and Tinker, 2000; Nishikawa et al., 1997). One of the major actions of GABA_B receptor activation is the opening of Kir3 channels, where the increase in K⁺ permeability through these channels hyperpolarises the cell thereby inhibiting the propagation of action potentials (Dascal, 1997; Misgeld et al., 1995).

Many GPCRs undergo rapid receptor phosphorylation and subsequent sequestration from the cell surface, commonly in an arrestin-dependent manner, followed by the recruitment of scaffolding proteins and by clathrin-mediated endocytosis (Shenoy and Lefkowitz, 2005). One interesting feature that is dissimilar to many GPCRs and is the subject of much debate is that GABA_B receptors do not appear to undergo activation-dependent phosphorylation and internalisation. Indeed, it has been reported that these receptors are not phosphorylated by the canonical G protein-coupled receptor kinases (GRKs), yet are desensitised by GRK4 in the absence of any apparent phosphorylation (Perroy et al., 2003). It has been demonstrated in chick neurons that upon activation, GABA_B receptors form a complex with Ca_v channels and arrestins, then are consequently internalised as a mechanism of rapid desensitisation of GABA_B receptor signalling (Puckerin et al., 2006). This however, is conflicting with evidence provided by Fairfax et al., (2004) whereby GABA_B receptors did not associate with arrestins and, indeed, the cAMP-dependent kinase- (PKA) mediated phosphorylation of the GABA_B receptor at position Ser892 on the GB₂ subunit increases its cell-surface stability; rather than impeding its cellular function. It would appear in these cases that the phosphorylation state and the subsequent events may very well be cell type specific, which may be yet another degree of complexity for texturing GABA_B receptor-mediated signalling. Interestingly, despite the lack of consistent evidence that GABA_B receptors are phosphorylated as a consequence of receptor activation, there is an accumulating body of evidence that these receptors are phosphorylated mostly by second-messenger kinases. For example, protein kinase C (PKC) has been described to phosphorylate the GB₁ subunit GABA_B receptors after the dissociation of the chaperone protein, *N*-ethylmaleimide-sensitive fusion (NSF) protein, in Chinese hamster ovary (CHO) cells (Pontier et al., 2006). More recently, there have been new developments on how GABA_B receptors are phosphorylated and dephosphorylated in neurons. Recent evidence suggests that NMDA receptors can also act as regulators of GABA_B receptor function, such that NMDA receptor activation, via calcium/calmodulin-dependent protein kinase, phosphorylates the GB₁ subunit at position Ser867, resulting in rapid receptor

internalisation from dendritic spines and shafts in the hippocampus (Guetg et al., 2010). Similarly, prolonged NMDA receptor activation results in the rapid phosphorylation of Ser783 on GB₂ in an 5' adenosine-monophosphate-dependent protein kinase- (AMPK) dependent manner (Terunuma et al., 2010). The rapid phosphorylation by AMPK altered the endocytic sorting pathway from receptor recycling to endosomal degradation, Ser783 was then slowly dephosphorylated by protein phosphatase 2A, returning the system back to its receptor recycling processes. Although the modes of which GABA_B receptors are phosphorylated and their consequences are not entirely clear, recently there has been a great deal of progress made in understanding how GABA_B receptor phosphorylation is affected by distinct signalling systems and their consequences on receptor function.

3.2 Localisation and physiology of GABA_B receptors

The GABA_B receptor is extensively expressed throughout the central nervous system, specifically, hippocampus, cortex, thalamus and cerebellum (Bettler and Tiao, 2006; Billinton et al., 1999); and in parts of the peripheral nervous system. They are located both pre- and post-synaptically where they mediate activity of Ca_v and Kir3 channels, respectively (Dutar and Nicoll, 1988; Lopez-Bendito et al., 2004; Luscher et al., 1997). Presynaptic GABA_B receptors can be found at both homo- and hetero-autoreceptors on GABA and, for example, glutamate nerve terminals, respectively (Thompson et al., 1993). Activation of these receptors leads to a hyperpolarisation of the nerve terminal thereby inhibiting further neurotransmitter release. Postsynaptically, GABA_B receptors have been demonstrated to mediate slow inhibitory postsynaptic potentials (IPSPs) through the operation of Kir3 channels. It is noteworthy that in the human brain, there are two major isoforms of the GABA_B receptors, those that contain a GB_{1A} subunits, and those that possess GB_{1B} subunits, notwithstanding there is no apparent difference in pharmacology or physiology between the two receptors in heterologous cell systems (Ulrich and Bettler, 2007). Despite a lack of obvious differences in function and pharmacology, there is indeed a differential expression pattern, such that GB_{1A} and GB_{1B} are both expressed on GABAergic nerve terminals, yet only GB_{1A} subunits are expressed on glutamatergic synaptic terminals (Kulik et al., 2003). By using different sets of complementary approaches, the authors showed that GB_{1A}-containing heterodimers mainly control presynaptic release of glutamate, whereas receptors possessing GB_{1B} subunits predominantly mediate post-synaptic inhibition.

3.3 GABA_B receptor pharmacology and clinical relevance

Similar to mGlu receptors, GABA_B receptors have two main ligand-binding domains, the orthosteric ligand-binding pocket located within the VFT of GB₁; and the allosteric ligand-binding domain, which is within the 7TM region, most likely within the 7TM bundle. There are surprisingly few GABA_B receptor full agonists aside from GABA itself and the well-known baclofen (refer to Figure 5). There are some other agonists such as CGP27492, the tritiated form of which replaced [³H]-baclofen as the radioligand agonist of choice, but was surrounded by controversy when it failed to reproduce the same physiological effects in some key assays (Froestl et al., 1995). A number of GABA_B receptor partial agonists have been identified, the most famous of which is the endogenous metabolite of GABA, γ -hydroxybutyric acid (GHB), synthesised from GABA transaminase and semialdehyde reductase. Other partial agonists include CGP44532 and CGP35024, the latter is also a

GABA_C receptor antagonist (Chebib et al., 1997). The number of antagonists is much greater than that of agonists, among these ligands there are the baclofen derivatives, saclofen and 2-OH saclofen; CGP54626, the most common of the antagonists; and CGP71872; the former two possessing high micromolar affinity, whilst the latter two exhibit low nanomolar affinity (Kaupmann et al., 1997).

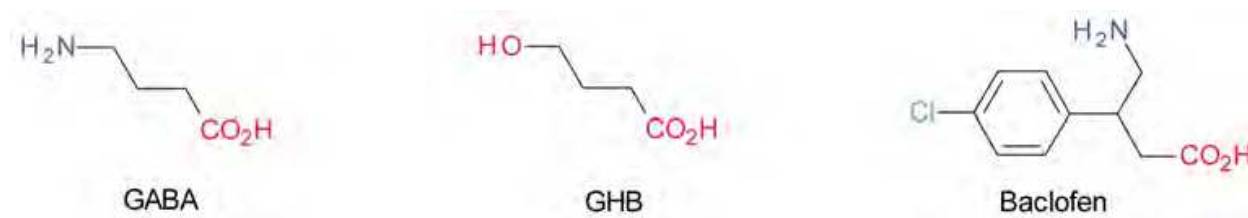


Fig. 5. Structural similarities across common GABA_B receptor agonists.

As with many Class C GPCRs, there exist a number of allosteric modulators available for the GABA_B receptor, yet all known modulators are PAMs, with no known NAMs, to date. Some PAMs of the GABA_B receptor are CGP7930, GS39783 and the more recent, rac-BHFF (Malherbe et al., 2008; Pin and Prezeau, 2007)(Figure 6). These PAMs increase orthosteric agonist potency and maximal response in a system-dependent manner, whilst possessing partial agonism in their own right. Given that many PAMs will most often on activate their target receptor when the endogenous or orthosteric ligand is present, they offer an ideal approach for drug discovery given they maintain region-dependent transmission patterns, therefore theoretically limiting off-target effects and side effect profile.

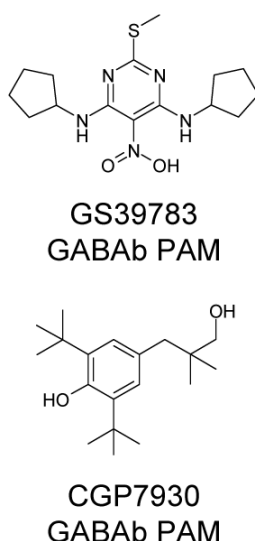


Fig. 6. Two of the best characterised positive allosteric modulators at the GABA_B receptor.

3.3.1 Addiction and GABA_B receptors

Today, there are two GABA_B receptor ligands on the market, both agonist, but both treat largely different disorders. Baclofen, originally developed to treat epilepsy in the 1920s, was largely unsuccessful for the treatment of epileptic symptoms, but its potential was realised outside of epileptic patients. Among the more common uses for baclofen is the treatment of addiction of abusive substances. Specifically, alcohol dependence has received much

attention with regard to GABAB receptors, such that baclofen administration in open-label trials reduced the number of heavy-drinking days and increased the number of abstinence days, in addition to decreasing biological markers such as alanine aminotransferase and gamma glutamyl-transpeptidase, in some patients (Addolorato et al., 2000; Flannery et al., 2004). Baclofen was not only useful for the management of alcohol addiction, but may also be employed as a strategy against withdrawal and relapse (Addolorato et al., 2006). When compared with treatment of diazepam, baclofen was only slightly less efficient at reducing the symptoms of alcohol withdrawal, such as sweating, anxiety and agitation; however this suggests baclofen may be a useful treatment for alcohol withdrawal in patients that abuse other substances, for example, benzodiazepines.

Baclofen has also been investigated for its effects on relieving addiction to cocaine. In one study, users of freebase or crack cocaine who self-administered through inhalation of the drug (Haney et al., 2006). Users who were either treated with methadone or not were given varying doses of baclofen and subsequently were asked to choose to take either the available dose of cocaine or five dollar merchandise voucher. The group who were administered 60mg of baclofen and non-methadone treated demonstrated a decrease in the craving for the low dose of cocaine (12mg), whilst there was no change in the methadone-treated group. Interestingly, baclofen also decreased the effect of cocaine on heart rate, however the personal evaluation of the 'high' remained unchanged. These results suggest that in some specific cases that baclofen would have a positive effect on addiction, however these situations are also often confounded by psychological dependence and are by and large heavily dictated by the patient.

3.3.2 GHB and current therapeutic indications

As previously mentioned, GHB is a minor metabolite of GABA; however in the 1960s GHB was first developed as a therapeutic as a CNS depressor (Laborit et al., 1960). At the time, it was also used as an adjuvant for anesthetics and is still used in some countries as an intravenous anesthesia (Kleinschmidt et al., 1997). Nowadays, the therapeutic indications for GHB are cataplexy and excessive daytime sleepiness associated with sleep disorder narcolepsy. Narcolepsy is the condition characterised by interrupted nighttime sleep and excessive daytime sleep, in addition to this, approximately 70% of narcoleptics suffer from cataplexy, which is a sudden loss of muscle tone. The evidence of clinical efficacy of GHB is largely empirical through a number of studies on narcoleptic patients, daily doses of GHB was able to reduce the number of nocturnal sleep/awake transitions, cataplexy episodes and the frequency between wakefulness and REM sleep during the daytime (Pardi and Black, 2006; Scrima et al., 1990). Despite clinical evidence supporting the therapeutic benefits of GHB for these conditions, there is still much debate over the molecular mechanism of action of GHB. There is known to be at least two GHB-binding sites, a high-affinity site on an unidentified protein; and a low-affinity site, which is at the GABA_B receptor (Kaupmann et al., 2003). However, there is evidence that the effects of GHB on stabilising patterns of somnolence are due to the subsequent actions at the GABA_B receptor. Recently, Vienne et al., (2010) provided evidence that the effects on somnolence and circadian sleep organisation are dependent on GABA_B receptors, whereby GHB and baclofen stabilised sleep/wake regulation in wild-type mice; these effects were lost in both GB₁^{-/-} and GB₂^{-/-} mice. This study suggests that the therapeutic benefits of GHB in narcoleptic patients may be mostly due to GHB-mediated activation of GABA_B receptors.

3.3.3 GABA_B receptors in pain

The importance of GABA_B receptors in nociceptive processing was well documented in the early 90's in a series of preclinical studies in which the GABA_B receptor agonist, baclofen, exhibited antinociceptive properties in models of acute (Malcangio et al., 1991) and chronic pain (Dirig and Yaksh, 1995; Smith et al., 1994). These effects are likely mediated by spinal and supraspinal GABA_B receptors; where the supraspinal effects appear to reflect depression of ascending adrenergic and dopaminergic input to the brainstem, and facilitation of descending noradrenergic input to the spinal cord dorsal horn (Sawynok, 1984). Baclofen-induced antinociception at spinal cord level is attributed, at least partly, to the activation of presynaptic GABA_B receptors localised on the nerve terminals of peptidergic primary afferents fibers (Price et al., 1984). In the substantia gelatinosa of the spinal cord, baclofen exhibits a greater effect on C-fibers than A δ -fiber-evoked glutamate release, suggesting a preferential GABA_B expression in C fibers afferent terminals (Ataka et al., 2000). Furthermore, baclofen inhibits electrically-evoked release of calcitonin gene-related peptide (CGRP) (Malcangio and Bowery, 1995) and substance P (Marvizon et al., 1999) from rat spinal cord slices. The decrease of dorsal horn neurons excitability and the regulation of intrinsic neuronal properties suggest additional postsynaptic sites for the action of baclofen on pain (Derjean et al., 2003; Kangrga et al., 1991). Taken together, the effects of activation of GABA_B receptors on the inhibition of pain signalling suggest that it is a tractable target for combating neuropathic and potentially other types of pain.

4. Concluding remarks

The treatment of neurological disorders is perhaps one of the most difficult tasks in modern day medicine; the multi-factorial nature of disease and the availability of appropriate therapeutics continually hamper the drug discovery process. The initial step in surmounting these obstacles is the validation of a target, which is perpetually being revised and, has now furnished two invaluable targets in the mGlu and GABA_B receptors. Both receptors, which present the major excitatory and inhibitory GPCR conduits, could be targeted for the treatment of a myriad of central and peripheral disorders. To better understand the function and physiology of these receptors it is paramount that we elucidate molecular mechanisms of receptor activation and ligand binding. There exists a large body of work the pharmacology of mGlu and GABA_B receptors, yet we are only now scratching the surface, as recently there has been an influx on novel receptor-selective pharmacophores, especially for mGlu receptors. With a better pharmacological armamentarium we will be better equipped to delineate (patho)physiological phenomena as we progress development of better therapeutics.

5. References

- Acher FC, Tellier FJ, Azerad R, Brabet IN, Fagni L and Pin JP (1997) Synthesis and pharmacological characterization of aminocyclopentanetricarboxylic acids: new tools to discriminate between metabotropic glutamate receptor subtypes. *J Med Chem* 40(19):3119-3129.

- Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL and Gasbarrini G (2000) Ability of baclofen in reducing alcohol craving and intake: II--Preliminary clinical evidence. *Alcohol Clin Exp Res* 24(1):67-71.
- Addolorato G, Leggio L, Abenavoli L, Agabio R, Caputo F, Capristo E, Colombo G, Gessa GL and Gasbarrini G (2006) Baclofen in the treatment of alcohol withdrawal syndrome: a comparative study vs diazepam. *Am J Med* 119(3):276 e213-278.
- Aghajanian GK and Marek GJ (1999) Serotonin and hallucinogens. *Neuropsychopharmacology* 21(2 Suppl):16S-23S.
- Agnati LF, Ferre S, Lluís C, Franco R and Fuxe K (2003) Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacol Rev* 55(3):509-550.
- Aiba A, Chen C, Herrup K, Rosenmund C, Stevens CF and Tonegawa S (1994a) Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* 79(2):365-375.
- Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, Zwingman TA and Tonegawa S (1994b) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 79(2):377-388.
- Allen SJ, Crown SE and Handel TM (2007) Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 25:787-820.
- Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND and Varney MA (2002) [3H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. *J Pharmacol Exp Ther* 303(3):1044-1051.
- Andrade L, Caraveo-Anduaga JJ, Berglund P, Bijl RV, De Graaf R, Vollebergh W, Dragomirecka E, Kohn R, Keller M, Kessler RC, Kawakami N, Kilic C, Offord D, Ustun TB and Wittchen HU (2003) The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. *Int J Methods Psychiatr Res* 12(1):3-21.
- Aniksztejn L, Otani S and Ben-Ari Y (1992) Quisqualate Metabotropic Receptors Modulate NMDA Currents and Facilitate Induction of Long-Term Potentiation Through Protein Kinase C. *Eur J Neurosci* 4(6):500-505.
- Annoura H, Fukunaga A, Uesugi M, Tatsuoka T and Horikawa Y (1996) A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylates. *Bioorg. Med. Chem. Lett.* 6, 763-766
- Antony J, Kellershohn K, Mohr-Andra M, Kebig A, Prilla S, Muth M, Heller E, Disingrini T, Dallanocce C, Bertoni S, Schrobang J, Trankle C, Kostenis E, Christopoulos A, Holtje HD, Barocelli E, De Amici M, Holzgrabe U and Mohr K (2009) Dualsteric GPCR targeting: a novel route to binding and signaling pathway selectivity. *Faseb J* 23(2):442-450.
- Ataka T, Kumamoto E, Shimoji K and Yoshimura M (2000) Baclofen inhibits more effectively C-afferent than Delta-afferent glutamatergic transmission in substantia gelatinosa neurons of adult rat spinal cord slices. *Pain* 86(3):273-282.

- Ayala JE, Niswender CM, Luo Q, Banko JL and Conn PJ (2008) Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated. *Neuropharmacology* 54(5):804-814.
- Azdad K, Gall D, Woods AS, Ledent C, Ferre S and Schiffmann SN (2009) Dopamine D2 and adenosine A2A receptors regulate NMDA-mediated excitation in accumbens neurons through A2A-D2 receptor heteromerization. *Neuropsychopharmacology* 34(4):972-986.
- Baptista MA, Martin-Fardon R and Weiss F (2004) Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. *J Neurosci* 24(20):4723-4727.
- Battaglia G, Busceti CL, Molinaro G, Biagioni F, Storto M, Fornai F, Nicoletti F and Bruno V (2004) Endogenous activation of mGlu5 metabotropic glutamate receptors contributes to the development of nigro-striatal damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *J Neurosci* 24(4):828-835.
- Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA and Somogyi P (1993) The metabotropic glutamate receptor (mGluR1 α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* 11(4):771-787.
- Belozertseva IV, Kos T, Popik P, Danysz W and Bessalov AY (2007) Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *Eur Neuropsychopharmacol* 17(3):172-179.
- Benneyworth MA, Smith RL and Sanders-Bush E (2008) Chronic phenethylamine hallucinogen treatment alters behavioral sensitivity to a metabotropic glutamate 2/3 receptor agonist. *Neuropsychopharmacology* 33(9):2206-2216.
- Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ and Sanders-Bush E (2007) A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. *Mol Pharmacol* 72(2):477-484.
- Berry-Kravis E, Hessel D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV and Hagerman R (2009) A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet* 46(4):266-271.
- Bessis AS, Bertrand HO, Galvez T, De Colle C, Pin JP and Acher F (2000) Three-dimensional model of the extracellular domain of the type 4a metabotropic glutamate receptor: new insights into the activation process. *Protein Sci* 9(11):2200-2209.
- Bessis AS, Rondard P, Gaven F, Brabet I, Triballeau N, Prezeau L, Acher F and Pin JP (2002) Closure of the Venus flytrap module of mGlu8 receptor and the activation process: Insights from mutations converting antagonists into agonists. *Proc Natl Acad Sci U S A* 99(17):11097-11102.
- Bettler B and Tiao JY (2006) Molecular diversity, trafficking and subcellular localization of GABAB receptors. *Pharmacol Ther* 110(3):533-543.
- Beurrier C, Lopez S, Revy D, Selvam C, Goudet C, Lherondel M, Gubellini P, Kerkerian-LeGoff L, Acher F, Pin JP and Amalric M (2009) Electrophysiological and behavioral evidence that modulation of metabotropic glutamate receptor 4 with a new agonist reverses experimental parkinsonism. *FASEB J* 23(10):3619-3628.

- Billinton A, Upton N and Bowery NG (1999) GABA(B) receptor isoforms GBR1a and GBR1b, appear to be associated with pre- and post-synaptic elements respectively in rat and human cerebellum. *Br J Pharmacol* 126(6):1387-1392.
- Bittencourt AS, Carobrez AP, Zamprogno LP, Tufik S and Schenberg LC (2004) Organization of single components of defensive behaviors within distinct columns of periaqueductal gray matter of the rat: role of N-methyl-D-aspartic acid glutamate receptors. *Neuroscience* 125(1):71-89.
- Bortolotto ZA, Fitzjohn SM and Collingridge GL (1999) Roles of metabotropic glutamate receptors in LTP and LTD in the hippocampus. *Curr Opin Neurobiol* 9(3):299-304.
- Bowers MS, McFarland K, Lake RW, Peterson YK, Lapish CC, Gregory ML, Lanier SM and Kalivas PW (2004) Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. *Neuron* 42(2):269-281.
- Brabet I, Parmentier ML, De Colle C, Bockaert J, Acher F and Pin JP (1998) Comparative effect of L-CCG-I, DCG-IV and gamma-carboxy-L-glutamate on all cloned metabotropic glutamate receptor subtypes. *Neuropharmacology* 37(8):1043-1051.
- Brauner-Osborne H, Wellendorph P and Jensen AA (2007) Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Curr Drug Targets* 8(1):169-184.
- Brody SA, Conquet F and Geyer MA (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur J Neurosci* 18(12):3361-3366.
- Brody SA, Dulawa SC, Conquet F and Geyer MA (2004) Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. *Mol Psychiatry* 9(1):35-41.
- Burrige K and Wennerberg K (2004) Rho and Rac take center stage. *Cell* 116(2):167-179.
- Bushell TJ, Sansig G, Collett VJ, van der Putten H and Collingridge GL (2002) Altered short-term synaptic plasticity in mice lacking the metabotropic glutamate receptor mGlu7. *ScientificWorldJournal* 2:730-737.
- Cabello N, Gandia J, Bertarelli DC, Watanabe M, Lluís C, Franco R, Ferre S, Lujan R and Ciruela F (2009) Metabotropic glutamate type 5, dopamine D2 and adenosine A2a receptors form higher-order oligomers in living cells. *J Neurochem* 109(5):1497-1507.
- Carroll FY, Stolle A, Beart PM, Voerste A, Brabet I, Mauler F, Joly C, Antonicek H, Bockaert J, Müller T, Pin JP and Prezeau L (2001) BAY36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol Pharmacol* 59(5):965-973.
- Chavez-Noriega LE, Schaffhauser H and Campbell UC (2002) Metabotropic glutamate receptors: potential drug targets for the treatment of schizophrenia. *Curr Drug Targets CNS Neurol Disord* 1(3):261-281.
- Chebib M, Vandenberg RJ, Froestl W and Johnston GA (1997) Unsaturated phosphinic analogues of gamma-aminobutyric acid as GABA(C) receptor antagonists. *Eur J Pharmacol* 329(2-3):223-229.
- Chen Y, Goudet C, Pin JP and Conn PJ (2008) N-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) acts through a novel site as a positive allosteric modulator of group 1 metabotropic glutamate receptors. *Mol Pharmacol* 73(3):909-918.

- Chojnacka-Wojcik E, Tatarczynska E and Pilc A (1997) The anxiolytic-like effect of metabotropic glutamate receptor antagonists after intrahippocampal injection in rats. *Eur J Pharmacol* 319(2-3):153-156.
- Christopoulos A and Kenakin T (2002) G protein-coupled receptor allostery and complexing. *Pharmacol Rev* 54(2):323-374.
- Ciruela F, Burgueno J, Casado V, Canals M, Marcellino D, Goldberg SR, Bader M, Fuxe K, Agnati LF, Lluís C, Franco R, Ferré S and Woods AS (2004) Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope-epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors. *Anal Chem* 76(18):5354-5363.
- Ciruela F, Escriche M, Burgueno J, Angulo E, Casado V, Soloviev MM, Canela EI, Mallol J, Chan WY, Lluís C, McIlhinney RA and Franco R (2001) Metabotropic glutamate 1α and adenosine A1 receptors assemble into functionally interacting complexes. *J Biol Chem* 276(21):18345-18351.
- Collingridge GL, Kehl SJ and McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J Physiol* 334:33-46.
- Combs-Agrar L, Kniazeff J, Norskov-Lauritsen L, Maurel D, Gassmann M, Gregor N, Prezeau L, Bettler B, Durroux T, Trinquet E and Pin JP (2011) The oligomeric state sets GABA(B) receptor signalling efficacy. *EMBO J* 30(12):2336-2349.
- Conn PJ, Christopoulos A and Lindsley CW (2009a) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov* 8(1):41-54.
- Conn PJ and Jones CK (2009) Promise of mGluR2/3 activators in psychiatry. *Neuropsychopharmacology* 34(1):248-249.
- Conn PJ, Lindsley CW and Jones CK (2009b) Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci* 30(1):25-31.
- Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* 37:205-237.
- Coyle JT (2006) Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol Neurobiol* 26(4-6):365-384.
- Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ and van Der Putten H (2003) Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. *Eur J Neurosci* 17(11):2409-2417.
- Dascal N (1997) Signalling via the G protein-activated K⁺ channels. *Cell Signal* 9(8):551-573.
- Depping AM, Komossa K, Kissling W and Leucht S (2010) Second-generation antipsychotics for anxiety disorders. *Cochrane Database Syst Rev*(12):CD008120.
- Derjean D, Bertrand S, Le Masson G, Landry M, Morisset V and Nagy F (2003) Dynamic balance of metabotropic inputs causes dorsal horn neurons to switch functional states. *Nat Neurosci* 6(3):274-281.
- Dirig DM and Yaksh TL (1995) Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *J Pharmacol Exp Ther* 275(1):219-227.
- Dolen G, Carpenter RL, Ocain TD and Bear MF (2010) Mechanism-based approaches to treating fragile X. *Pharmacol Ther* 127(1):78-93.

- Doumazane E, Scholler P, Zwier JM, Eric T, Rondard P and Pin JP (2011) A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. *FASEB J* 25(1):66-77.
- Doupnik CA (2008) GPCR-Kir channel signaling complexes: defining rules of engagement. *J Recept Signal Transduct Res* 28(1-2):83-91.
- Dunayevich E, Erickson J, Levine L, Landbloom R, Schoepp DD and Tollefson GD (2008) Efficacy and tolerability of an mGlu2/3 agonist in the treatment of generalized anxiety disorder. *Neuropsychopharmacology* 33(7):1603-1610.
- Dunlap K and Fischbach GD (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J Physiol* 317:519-535.
- Dutar P and Nicoll RA (1988) Pre- and postsynaptic GABAB receptors in the hippocampus have different pharmacological properties. *Neuron* 1(7):585-591.
- Duty S (2010) Therapeutic potential of targeting group III metabotropic glutamate receptors in the treatment of Parkinson's disease. *Br J Pharmacol* 161(2):271-287.
- Duvoisin RM, Zhang C, Pfankuch TF, O'Connor H, Gayet-Primo J, Quraishi S and Raber J (2005) Increased measures of anxiety and weight gain in mice lacking the group III metabotropic glutamate receptor mGluR8. *Eur J Neurosci* 22(2):425-436.
- Engin E and Treit D (2008) The effects of intra-cerebral drug infusions on animals' unconditioned fear reactions: a systematic review. *Prog Neuropsychopharmacol Biol Psychiatry* 32(6):1399-1419.
- Fairfax BP, Pitcher JA, Scott MG, Calver AR, Pangalos MN, Moss SJ and Couve A (2004) Phosphorylation and chronic agonist treatment atypically modulate GABAB receptor cell surface stability. *J Biol Chem* 279(13):12565-12573.
- Fell MJ, Svensson KA, Johnson BG and Schoepp DD (2008) Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039). *J Pharmacol Exp Ther* 326(1):209-217.
- Felts AS, Lindsley SR, Lamb JP, Rodriguez AL, Menon UN, Jadhav S, Jones CK, Conn PJ, Lindsley CW and Emmitte KA (2010) 3-Cyano-5-fluoro-N-arylbenzamides as negative allosteric modulators of mGlu(5): Identification of easily prepared tool compounds with CNS exposure in rats. *Bioorg Med Chem Lett* 20(15):4390-4394.
- Ferraguti F and Shigemoto R (2006) Metabotropic glutamate receptors. *Cell Tissue Res* 326(2):483-504.
- Ferre S, Ciruela F, Quiroz C, Lujan R, Popoli P, Cunha RA, Agnati LF, Fuxe K, Woods AS, Lluís C and Franco R (2007) Adenosine receptor heteromers and their integrative role in striatal function. *ScientificWorldJournal* 7:74-85.
- Ferre S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, Casado V, Fuxe K, Goldberg SR, Lluís C, Franco R and Ciruela F (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. *Proc Natl Acad Sci U S A* 99(18):11940-11945.

- Feyissa AM, Chandran A, Stockmeier CA and Karolewicz B (2009) Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 33(1):70-75.
- Flannery BA, Garbutt JC, Cody MW, Renn W, Grace K, Osborne M, Crosby K, Morreale M and Trivette A (2004) Baclofen for alcohol dependence: a preliminary open-label study. *Alcohol Clin Exp Res* 28(10):1517-1523.
- Fredriksson R, Lagerstrom MC, Lundin LG and Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 63(6):1256-1272.
- Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J, Olpe HR and et al. (1995) Phosphinic acid analogues of GABA. 1. New potent and selective GABAB agonists. *J Med Chem* 38(17):3297-3312.
- Galandrin S, Oligny-Longpre G and Bouvier M (2007) The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci* 28(8):423-430.
- Galici R, Jones CK, Hemstapat K, Nong Y, Echemendia NG, Williams LC, de Paulis T and Conn PJ (2006) Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. *J Pharmacol Exp Ther* 318(1):173-185.
- Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prezeau L and Pin JP (2001) Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO J* 20(9):2152-2159.
- Gasparini F, Lingenhoehl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacca AI, Santori EM, Velicelebi G and Kuhn R (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* 38(10):1493-1503.
- Gerlai R, Roder JC and Hampson DR (1998) Altered spatial learning and memory in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *Behav Neurosci* 112(3):525-532.
- Ghasemzadeh MB, Mueller C and Vasudevan P (2009) Behavioral sensitization to cocaine is associated with increased glutamate receptor trafficking to the postsynaptic density after extended withdrawal period. *Neuroscience* 159(1):414-426.
- Gonzalez-Maesó J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ and Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 452(7183):93-97.
- Gonzalez-Maesó J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q, Sealfon SC and Gingrich JA (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* 53(3):439-452.
- Gonzalez-Maesó J, Yuen T, Ebersole BJ, Wurmbach E, Lira A, Zhou M, Weisstaub N, Hen R, Gingrich JA and Sealfon SC (2003) Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex. *J Neurosci* 23(26):8836-8843.

- Goudet C, Gaven F, Kniazeff J, Vol C, Liu J, Cohen-Gonsaud M, Acher F, Prezeau L and Pin JP (2004) Heptahelical domain of metabotropic glutamate receptor 5 behaves like rhodopsin-like receptors. *Proc Natl Acad Sci U S A* 101(1):378-383.
- Guettg N, Abdel Aziz S, Holbro N, Turecek R, Rose T, Seddik R, Gassmann M, Moes S, Jenoe P, Oertner TG, Casanova E and Bettler B (2010) NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. *Proc Natl Acad Sci U S A* 107(31):13924-13929.
- Hammond AS, Rodriguez AL, Townsend SD, Niswender CM, Gregory KJ, Lindsley CW and Conn PJ (2010) Discovery of a Novel Chemical Class of mGlu(5) Allosteric Ligands with Distinct Modes of Pharmacology. *ACS Chem Neurosci* 1(10):702-716.
- Haney M, Hart CL and Foltin RW (2006) Effects of baclofen on cocaine self-administration: opioid- and nonopioid-dependent volunteers. *Neuropsychopharmacology* 31(8):1814-1821.
- Hanyaloglu AC and von Zastrow M (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. *Annu Rev Pharmacol Toxicol* 48:537-568.
- Harvey J and Collingridge GL (1993) Signal transduction pathways involved in the acute potentiation of NMDA responses by 1S,3R-ACPD in rat hippocampal slices. *Br J Pharmacol* 109(4):1085-1090.
- Hemstapat K, de Paulis T, Chen Y, Brady AE, Grover VK, Alagille D, Tamagnan GD and Conn PJ (2006) A novel class of positive allosteric modulators of metabotropic glutamate receptor subtype 1 interact with a site distinct from that of negative allosteric modulators. *Mol Pharmacol* 70(2):616-626.
- Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T and Catterall WA (1996) Modulation of Ca²⁺ channels by G-protein beta gamma subunits. *Nature* 380(6571):258-262.
- Hirono M, Yoshioka T and Konishi S (2001) GABA(B) receptor activation enhances mGluR-mediated responses at cerebellar excitatory synapses. *Nat Neurosci* 4(12):1207-1216.
- Hirsch EC (2000) Nigrostriatal system plasticity in Parkinson's disease: effect of dopaminergic denervation and treatment. *Ann Neurol* 47(4 Suppl 1):S115-120; discussion S120-111.
- Hoare SR (2005) Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors. *Drug Discov Today* 10(6):417-427.
- Hu J, Hauache O and Spiegel AM (2000) Human Ca²⁺ receptor cysteine-rich domain. Analysis of function of mutant and chimeric receptors. *J Biol Chem* 275(21):16382-16389.
- Jiang P, Ji Q, Liu Z, Snyder LA, Benard LM, Margolskee RF and Max M (2004) The cysteine-rich region of T1R3 determines responses to intensely sweet proteins. *J Biol Chem* 279(43):45068-45075.
- Johnson MP, Baez M, Jagdmann GE, Jr., Britton TC, Large TH, Callagaro DO, Tizzano JP, Monn JA and Schoepp DD (2003) Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine. *J Med Chem* 46(15):3189-3192.

- Kachroo A, Orlando LR, Grandy DK, Chen JF, Young AB and Schwarzschild MA (2005) Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. *J Neurosci* 25(45):10414-10419.
- Kammerer RA, Frank S, Schulthess T, Landwehr R, Lustig A and Engel J (1999) Heterodimerization of a functional GABAB receptor is mediated by parallel coiled-coil alpha-helices. *Biochemistry* 38(40):13263-13269.
- Kangrga I, Jiang MC and Randic M (1991) Actions of (-)-baclofen on rat dorsal horn neurons. *Brain Res* 562(2):265-275.
- Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Brauner-Osborne H, Waldmeier P and Bettler B (2003) Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. *Eur J Neurosci* 18(10):2722-2730.
- Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 386(6622):239-246.
- Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A and Bettler B (1998) GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* 396(6712):683-687.
- Kenny PJ and Markou A (2004) The ups and downs of addiction: role of metabotropic glutamate receptors. *Trends Pharmacol Sci* 25(5):265-272.
- Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, Tiller PR, Smith S, Jacobson MA, Sur C, Duggan ME, Pettibone DJ, Conn PJ and Williams DL, Jr. (2005) A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther* 313(1):199-206.
- Kleinschmidt S, Grundmann U, Janneck U, Kreienmeyer J, Kulosa R and Larsen R (1997) Total intravenous anaesthesia using propofol, gamma-hydroxybutyrate or midazolam in combination with sufentanil for patients undergoing coronary artery bypass surgery. *Eur J Anaesthesiol* 14(6):590-599.
- Klodzinska A, Tatarczynska E, Stachowicz K and Chojnacka-Wojcik E (2004) The anxiolytic-like activity of AIDA (1-aminoindan-1,5-dicarboxylic acid), an mGlu 1 receptor antagonist. *J Physiol Pharmacol* 55(1 Pt 1):113-126.
- Kniazeff J, Galvez T, Labesse G and Pin JP (2002) No ligand binding in the GB2 subunit of the GABA(B) receptor is required for activation and allosteric interaction between the subunits. *J Neurosci* 22(17):7352-7361.
- Kniazeff J, Prezeau L, Rondard P, Pin JP and Goudet C (2011) Dimers and beyond: The functional puzzles of class C GPCRs. *Pharmacol Ther* 130(1):9-25.
- Knoflach F, Mutel V, Jolidon S, Kew JN, Malherbe P, Vieira E, Wichmann J and Kemp JA (2001) Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc Natl Acad Sci U S A* 98(23):13402-13407.

- Kolber BJ, Montana MC, Carrasquillo Y, Xu J, Heinemann SF, Muglia LJ and Gereau RWt (2010) Activation of metabotropic glutamate receptor 5 in the amygdala modulates pain-like behavior. *J Neurosci* 30(24):8203-8213.
- Kulik A, Vida I, Lujan R, Haas CA, Lopez-Bendito G, Shigemoto R and Frotscher M (2003) Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *J Neurosci* 23(35):11026-11035.
- Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H and Morikawa K (2000) Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 407(6807):971-977.
- Laborit H, Jouany JM, Gerard J and Fabiani F (1960) [Summary of an experimental and clinical study on a metabolic substrate with inhibitory central action: sodium 4-hydroxybutyrate]. *Presse Med* 68:1867-1869.
- Leaney JL and Tinker A (2000) The role of members of the pertussis toxin-sensitive family of G proteins in coupling receptors to the activation of the G protein-gated inwardly rectifying potassium channel. *Proc Natl Acad Sci U S A* 97(10):5651-5656.
- Lefkowitz RJ (1998) G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. *J Biol Chem* 273(30):18677-18680.
- Lehmann A (2008) Novel treatments of GERD: focus on the lower esophageal sphincter. *Eur Rev Med Pharmacol Sci* 12 Suppl 1:103-110.
- Linden AM, Shannon H, Baez M, Yu JL, Koester A and Schoepp DD (2005) Anxiolytic-like activity of the mGlu2/3 receptor agonist LY354740 in the elevated plus maze test is disrupted in metabotropic glutamate receptor 2 and 3 knock-out mice. *Psychopharmacology (Berl)* 179(1):284-291.
- Litschig S, Gasparini F, Rueegg D, Stoehr N, Flor PJ, Vranesic I, Prezeau L, Pin JP, Thomsen C and Kuhn R (1999) CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* 55(3):453-461.
- Lopez S, Turle-Lorenzo N, Acher F, De Leonibus E, Mele A and Amalric M (2007) Targeting group III metabotropic glutamate receptors produces complex behavioral effects in rodent models of Parkinson's disease. *J Neurosci* 27(25):6701-6711.
- Lopez-Bendito G, Shigemoto R, Kulik A, Vida I, Fairen A and Lujan R (2004) Distribution of metabotropic GABA receptor subunits GABAB1a/b and GABAB2 in the rat hippocampus during prenatal and postnatal development. *Hippocampus* 14(7):836-848.
- Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM and Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci* 17(13):5196-5205.
- Luscher C, Jan LY, Stoffel M, Malenka RC and Nicoll RA (1997) G protein-coupled inwardly rectifying K⁺ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* 19(3):687-695.
- Maj M, Bruno V, Dragic Z, Yamamoto R, Battaglia G, Inderbitzin W, Stoehr N, Stein T, Gasparini F, Vranesic I, Kuhn R, Nicoletti F and Flor PJ (2003) (-)-PHCCC, a

- positive allosteric modulator of mGluR4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* 45(7):895-906.
- Malcangio M and Bowery NG (1995) Possible therapeutic application of GABAB receptor agonists and antagonists. *Clin Neuropharmacol* 18(4):285-305.
- Malcangio M, Ghelardini C, Giotti A, Malmberg-Aiello P and Bartolini A (1991) CGP 35348, a new GABAB antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen. *Br J Pharmacol* 103(2):1303-1308.
- Malherbe P, Masciadri R, Norcross RD, Knoflach F, Kratzeisen C, Zenner MT, Kolb Y, Marcuz A, Huwyler J, Nakagawa T, Porter RH, Thomas AW, Wettstein JG, Sleight AJ, Spooren W and Prinssen EP (2008) Characterization of (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABAB receptors. *Br J Pharmacol* 154(4):797-811.
- Margeta-Mitrovic M, Jan YN and Jan LY (2001) Function of GB1 and GB2 subunits in G protein coupling of GABA(B) receptors. *Proc Natl Acad Sci U S A* 98(25):14649-14654.
- Martin LJ, Blackstone CD, Huganir RL and Price DL (1992) Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron* 9(2):259-270.
- Marvizon JC, Grady EF, Stefani E, Bunnett NW and Mayer EA (1999) Substance P release in the dorsal horn assessed by receptor internalization: NMDA receptors counteract a tonic inhibition by GABA(B) receptors. *Eur J Neurosci* 11(2):417-426.
- Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R and et al. (1995) Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell* 80(5):757-765.
- Maurel D, Comps-Agrar L, Brock C, Rives ML, Bourrier E, Ayoub MA, Bazin H, Tinel N, Durroux T, Prezeau L, Trinquet E and Pin JP (2008) Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. *Nat Methods* 5(6):561-567.
- Miedlich SU, Gama L, Seuwen K, Wolf RM and Breitwieser GE (2004) Homology modeling of the transmembrane domain of the human calcium sensing receptor and localization of an allosteric binding site. *J Biol Chem* 279(8):7254-7263.
- Misgeld U, Bijak M and Jarolimek W (1995) A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system. *Prog Neurobiol* 46(4):423-462.
- Molina-Hernandez M, Tellez-Alcantara NP, Perez-Garcia J, Olivera-Lopez JI and Jaramillo-Jaimes MT (2008) Antidepressant-like actions of minocycline combined with several glutamate antagonists. *Prog Neuropsychopharmacol Biol Psychiatry* 32(2):380-386.
- Morishima Y, Miyakawa T, Furuyashiki T, Tanaka Y, Mizuma H and Nakanishi S (2005) Enhanced cocaine responsiveness and impaired motor coordination in metabotropic glutamate receptor subtype 2 knockout mice. *Proc Natl Acad Sci U S A* 102(11):4170-4175.
- Moussawi K and Kalivas PW (2010) Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. *Eur J Pharmacol* 639(1-3):115-122.
- Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A and Kalivas PW (2009) N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci* 12(2):182-189.

- Muto T, Tsuchiya D, Morikawa K and Jingami H (2007) Expression, purification, crystallization and preliminary X-ray analysis of the ligand-binding domain of metabotropic glutamate receptor 7. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 63(Pt 7):627-630.
- Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N and Nakanishi S (1993) Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J Biol Chem* 268(16):11868-11873.
- Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ and Zuker CS (2001) Mammalian sweet taste receptors. *Cell* 106(3):381-390.
- Neugebauer V, Zinebi F, Russell R, Gallagher JP and Shinnick-Gallagher P (2000) Cocaine and kindling alter the sensitivity of group II and III metabotropic glutamate receptors in the central amygdala. *J Neurophysiol* 84(2):759-770.
- Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT and Pin JP (2011) Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology* 60(7-8):1017-1041.
- Nishikawa M, Hirouchi M and Kuriyama K (1997) Functional coupling of Gi subtype with GABAB receptor/adenylyl cyclase system: analysis using a reconstituted system with purified GTP-binding protein from bovine cerebral cortex. *Neurochem Int* 31(1):21-25.
- Niswender CM and Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50:295-322.
- Niswender CM, Johnson KA, Miller NR, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD and Conn PJ (2010) Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol* 77(3):459-468.
- Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, Days EL, Nalywajko T, Austin CA, Williams MB, Ayala JE, Williams R, Lindsley CW and Conn PJ (2008) Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Mol Pharmacol* 74(5):1345-1358.
- O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL and Mulvihill ER (1993) The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* 11(1):41-52.
- Ohishi H, Neki A and Mizuno N (1998) Distribution of a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat and mouse: an immunohistochemical study with a monoclonal antibody. *Neurosci Res* 30(1):65-82.
- Ohishi H, Ogawa-Meguro R, Shigemoto R, Kaneko T, Nakanishi S and Mizuno N (1994) Immunohistochemical localization of metabotropic glutamate receptors, mGluR2 and mGluR3, in rat cerebellar cortex. *Neuron* 13(1):55-66.
- Oliet SH, Malenka RC and Nicoll RA (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. *Neuron* 18(6):969-982.

- Ossowska K, Pietraszek M, Wardas J and Wolfarth S (2004) Potential antipsychotic and extrapyramidal effects of (R,S)-3,4-dicarboxyphenylglycine [(R,S)-3,4-DCPG], a mixed AMPA antagonist/mGluR8 agonist. *Pol J Pharmacol* 56(3):295-304.
- Overington JP, Al-Lazikani B and Hopkins AL (2006) How many drug targets are there? *Nat Rev Drug Discov* 5(12):993-996.
- Pagano A, Ruegg D, Litschig S, Stoehr N, Stierlin C, Heinrich M, Floersheim P, Prezeau L, Carroll F, Pin JP, Cambria A, Vranesic I, Flor PJ, Gasparini F and Kuhn R (2000) The non-competitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of group I metabotropic glutamate receptors. *J Biol Chem* 275(43):33750-33758.
- Palucha A and Pilc A (2005) The involvement of glutamate in the pathophysiology of depression. *Drug News Perspect* 18(4):262-268.
- Pardi D and Black J (2006) gamma-Hydroxybutyrate/sodium oxybate: neurobiology, and impact on sleep and wakefulness. *CNS Drugs* 20(12):993-1018.
- Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA and Schoepp DD (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nat Med* 13(9):1102-1107.
- Paul IA and Skolnick P (2003) Glutamate and depression: clinical and preclinical studies. *Ann N Y Acad Sci* 1003:250-272.
- Pekhletski R, Gerlai R, Overstreet LS, Huang XP, Agopyan N, Slater NT, Abramow-Newerly W, Roder JC and Hampson DR (1996) Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *J Neurosci* 16(20):6364-6373.
- Peleg S, Varon D, Ivanina T, Dessauer CW and Dascal N (2002) G(alpha)(i) controls the gating of the G protein-activated K(+) channel, GIRK. *Neuron* 33(1):87-99.
- Perroy J, Adam L, Qanbar R, Chenier S and Bouvier M (2003) Phosphorylation-independent desensitization of GABA(B) receptor by GRK4. *EMBO J* 22(15):3816-3824.
- Perroy J, Raynaud F, Homburger V, Rousset MC, Telley L, Bockaert J and Fagni L (2008) Direct interaction enables cross-talk between ionotropic and group I metabotropic glutamate receptors. *J Biol Chem* 283(11):6799-6805.
- Petersen EN and Lassen JB (1981) A water lick conflict paradigm using drug experienced rats. *Psychopharmacology (Berl)* 75(3):236-239.
- Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D and Ruat M (2004) Positive and negative allosteric modulators of the Ca²⁺-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. *J Biol Chem* 279(18):18990-18997.
- Pin JP and Acher F (2002) The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr Drug Targets CNS Neurol Disord* 1(3):297-317.
- Pin JP, Kniazeff J, Goudet C, Bessis AS, Liu J, Galvez T, Acher F, Rondard P and Prezeau L (2004) The activation mechanism of class-C G-protein coupled receptors. *Biol Cell* 96(5):335-342.

- Pin JP and Prezeau L (2007) Allosteric modulators of GABA(B) receptors: mechanism of action and therapeutic perspective. *Curr Neuropharmacol* 5(3):195-201.
- Pisani A, Bonsi P, Centonze D, Gubellini P, Bernardi G and Calabresi P (2003) Targeting striatal cholinergic interneurons in Parkinson's disease: focus on metabotropic glutamate receptors. *Neuropharmacology* 45(1):45-56.
- Pontier SM, Lahaie N, Gingham R, St-Gelais F, Bonin H, Bell DJ, Flynn H, Trudeau LE, McIlhinney J, White JH and Bouvier M (2006) Coordinated action of NSF and PKC regulates GABAB receptor signaling efficacy. *EMBO J* 25(12):2698-2709.
- Price DD, Rafii A, Watkins LR and Buckingham B (1984) A psychophysical analysis of acupuncture analgesia. *Pain* 19(1):27-42.
- Puckerin A, Liu L, Permaul N, Carman P, Lee J and Diverse-Pierluissi MA (2006) Arrestin is required for agonist-induced trafficking of voltage-dependent calcium channels. *J Biol Chem* 281(41):31131-31141.
- Rondard P, Liu J, Huang S, Malhaire F, Vol C, Pinault A, Labesse G and Pin JP (2006) Coupling of agonist binding to effector domain activation in metabotropic glutamate-like receptors. *J Biol Chem* 281(34):24653-24661.
- Sansig G, Bushell TJ, Clarke VR, Rozov A, Burnashev N, Portet C, Gasparini F, Schmutz M, Klebs K, Shigemoto R, Flor PJ, Kuhn R, Knoepfel T, Schroeder M, Hampson DR, Collett VJ, Zhang C, Duvoisin RM, Collingridge GL and van Der Putten H (2001) Increased seizure susceptibility in mice lacking metabotropic glutamate receptor 7. *J Neurosci* 21(22):8734-8745.
- Sato M, Horinouchi T, Hutchinson DS, Evans BA and Summers RJ (2007) Ligand-directed signaling at the beta3-adrenoceptor produced by 3-(2-Ethylphenoxy)-1-[(1S)-1,2,3,4-tetrahydronaph-1-ylamino]-2S-2-propan ol oxalate (SR59230A) relative to receptor agonists. *Mol Pharmacol* 72(5):1359-1368.
- Sawynok J (1984) GABAergic mechanisms in antinociception. *Prog Neuropsychopharmacol Biol Psychiatry* 8(4-6):581-586.
- Schaffhauser H, Knoflach F, Pink JR, Bleuel Z, Cartmell J, Goepfert F, Kemp JA, Richards JG, Adam G and Mutel V (1998) Multiple pathways for regulation of the KCl-induced [3H]-GABA release by metabotropic glutamate receptors, in primary rat cortical cultures. *Brain Res* 782(1-2):91-104.
- Schann S, Menet C, Arvault P, Mercier G, Frauli M, Mayer S, Hubert N, Triballeau N, Bertrand HO, Acher F and Neuville P (2006) Design and synthesis of APTCs (aminopyrrolidinetricarboxylic acids): identification of a new group III metabotropic glutamate receptor selective agonist. *Bioorg Med Chem Lett* 16(18):4856-4860.
- Scholten D, Canals M, Maussang D, Roumen L, Smit M, Wijtmans M, de Graaf C, Vischer H and Leurs R (2011) Pharmacological Modulation of Chemokine Receptor Function. *Br J Pharmacol*.
- Schweitzer C, Kratzeisen C, Adam G, Lundstrom K, Malherbe P, Ohresser S, Stadler H, Wichmann J, Woltering T and Mutel V (2000) Characterization of [(3)H]-LY354740 binding to rat mGlu2 and mGlu3 receptors expressed in CHO cells using semliki forest virus vectors. *Neuropharmacology* 39(10):1700-1706.

- Scrima L, Hartman PG, Johnson FH, Jr., Thomas EE and Hiller FC (1990) The effects of gamma-hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. *Sleep* 13(6):479-490.
- Selvam C, Oueslati N, Lemasson IA, Brabet I, Rigault D, Courtiol T, Cesarini S, Triballeau N, Bertrand HO, Goudet C, Pin JP and Acher FC (2010) A virtual screening hit reveals new possibilities for developing group III metabotropic glutamate receptor agonists. *J Med Chem* 53(7):2797-2813.
- Sexton PM, Morfis M, Tilakaratne N, Hay DL, Udawela M, Christopoulos G and Christopoulos A (2006) Complexing receptor pharmacology: modulation of family B G protein-coupled receptor function by RAMPs. *Ann N Y Acad Sci* 1070:90-104.
- Shenoy SK and Lefkowitz RJ (2005) Receptor regulation: beta-arrestin moves up a notch. *Nat Cell Biol* 7(12):1159-1161.
- Shimazaki T, Iijima M and Chaki S (2004) Anxiolytic-like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test. *Eur J Pharmacol* 501(1-3):121-125.
- Smith GD, Harrison SM, Birch PJ, Elliott PJ, Malcangio M and Bowery NG (1994) Increased sensitivity to the antinociceptive activity of (+/-)-baclofen in an animal model of chronic neuropathic, but not chronic inflammatory hyperalgesia. *Neuropharmacology* 33(9):1103-1108.
- Steckler T, Lavreysen H, Oliveira AM, Aerts N, Van Craenendonck H, Prickaerts J, Megens A and Lesage AS (2005) Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. *Psychopharmacology (Berl)* 179(1):198-206.
- Sudo S, Kumagai J, Nishi S, Layfield S, Ferraro T, Bathgate RA and Hsueh AJ (2003) H3 relaxin is a specific ligand for LGR7 and activates the receptor by interacting with both the ectodomain and the exoloop 2. *J Biol Chem* 278(10):7855-7862.
- Sung KW, Choi S and Lovinger DM (2001) Activation of group I mGluRs is necessary for induction of long-term depression at striatal synapses. *J Neurophysiol* 86(5):2405-2412.
- Suzuki G, Kimura T, Satow A, Kaneko N, Fukuda J, Hikichi H, Sakai N, Maehara S, Kawagoe-Takaki H, Hata M, Azuma T, Ito S, Kawamoto H and Ohta H (2007) Pharmacological characterization of a new, orally active and potent allosteric metabotropic glutamate receptor 1 antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC). *J Pharmacol Exp Ther* 321(3):1144-1153.
- Tamaru Y, Nomura S, Mizuno N and Shigemoto R (2001) Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. *Neuroscience* 106(3):481-503.
- Tebano MT, Martire A, Pepponi R, Domenici MR and Popoli P (2006) 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* 2(4):619-625.
- Terunuma M, Vargas KJ, Wilkins ME, Ramirez OA, Jaureguierry-Bravo M, Pangalos MN, Smart TG, Moss SJ and Couve A (2010) Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proc Natl Acad Sci U S A* 107(31):13918-13923.

- Thompson SM, Capogna M and Scanziani M (1993) Presynaptic inhibition in the hippocampus. *Trends Neurosci* 16(6):222-227.
- Toms NJ and Roberts PJ (1999) Group 1 mGlu receptors elevate $[Ca^{2+}]_i$ in rat cultured cortical type 2 astrocytes: $[Ca^{2+}]_i$ synergy with adenosine A1 receptors. *Neuropharmacology* 38(10):1511-1517.
- Trullas R and Skolnick P (1990) Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol* 185(1):1-10.
- Tsuchiya D, Kunishima N, Kamiya N, Jingami H and Morikawa K (2002) Structural views of the ligand-binding cores of a metabotropic glutamate receptor complexed with an antagonist and both glutamate and Gd^{3+} . *Proc Natl Acad Sci U S A* 99(5):2660-2665.
- Tyacke RJ, Lingford-Hughes A, Reed LJ and Nutt DJ (2010) GABAB receptors in addiction and its treatment. *Adv Pharmacol* 58:373-396.
- Ulrich D and Bettler B (2007) GABA(B) receptors: synaptic functions and mechanisms of diversity. *Curr Opin Neurobiol* 17(3):298-303.
- Urizar E, Yano H, Kolster R, Gales C, Lambert N and Javitch JA (2011) CODA-RET reveals functional selectivity as a result of GPCR heteromerization. *Nat Chem Biol* 7(9):624-630.
- Urwiler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B and Kaupmann K (2001) Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* 60(5):963-971.
- Valant C, Gregory KJ, Hall NE, Scammells PJ, Lew MJ, Sexton PM and Christopoulos A (2008) A novel mechanism of G protein-coupled receptor functional selectivity. Muscarinic partial agonist McN-A-343 as a bitopic orthosteric/allosteric ligand. *J Biol Chem* 283(43):29312-29321.
- Valant C, Sexton PM and Christopoulos A (2009) Orthosteric/allosteric bitopic ligands: going hybrid at GPCRs. *Mol Interv* 9(3):125-135.
- Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG and Conn PJ (2003) Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. *J Neurosci* 23(18):7218-7226.
- Vienne J, Bettler B, Franken P and Tafti M (2010) Differential effects of GABAB receptor subtypes, γ -hydroxybutyric Acid, and Baclofen on EEG activity and sleep regulation. *J Neurosci* 30(42):14194-14204.
- Wettschureck N and Offermanns S (2005) Mammalian G proteins and their cell type specific functions. *Physiol Rev* 85(4):1159-1204.
- White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM and Marshall FH (1998) Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* 396(6712):679-682.
- Woolley ML, Pemberton DJ, Bate S, Corti C and Jones DN (2008) The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. *Psychopharmacology (Berl)* 196(3):431-440.

- Wright RA, Arnold MB, Wheeler WJ, Ornstein PL and Schoepp DD (2000) Binding of [3H](2S,1'S,2'S)-2-(9-xanthylmethyl)-2-(2'-carboxycyclopropyl) glycine ([3H]LY341495) to cell membranes expressing recombinant human group III metabotropic glutamate receptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* 362(6):546-554.
- Yamaguchi S and Ninomiya K (2000) Umami and food palatability. *J Nutr* 130(4S Suppl):921S-926S.
- Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C and Li X (2008) Molecular mechanism for the umami taste synergism. *Proc Natl Acad Sci U S A* 105(52):20930-20934.



Pharmacology

Edited by Dr. Luca Gallelli

ISBN 978-953-51-0222-9

Hard cover, 720 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

The history of pharmacology travels together to history of scientific method and the latest frontiers of pharmacology open a new world in the search of drugs. New technologies and continuing progress in the field of pharmacology has also changed radically the way of designing a new drug. In fact, modern drug discovery is based on deep knowledge of the disease and of both cellular and molecular mechanisms involved in its development. The purpose of this book was to give a new idea from the beginning of the pharmacology, starting from pharmacodynamic and reaching the new field of pharmacogenetic and ethnopharmacology.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Gregory Stewart, Julie Kniazeff, Laurent Prézeau, Philippe Rondard, Jean-Philippe Pin and Cyril Goudet (2012). Metabotropic Receptors for Glutamate and GABA, *Pharmacology*, Dr. Luca Gallelli (Ed.), ISBN: 978-953-51-0222-9, InTech, Available from: <http://www.intechopen.com/books/pharmacology/metabotropic-receptors-for-glutamate-and-gaba>

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