British Journal of Pharmacology (2009), 156, 869–884

0007-1188/09
www.brjpharmacol.org

REVIEW

Molecular basis for amino acid sensing by family C G-protein-coupled receptors

P Wellendorph and H Bräuner-Osborne

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

Family C of human G-protein-coupled receptors (GPCRs) is constituted by eight metabotropic glutamate receptors, two γ-aminobutyric acid type B ($GABA_{B1-2}$) subunits forming the heterodimeric $GABA_B$ receptor, the calcium-sensing receptor, three taste1 receptors (T1R1–3), a promiscuous L-α-amino acid receptor G-protein-coupled receptor family C, group 6, subtype A (GPRC6A) and seven orphan receptors. Aside from the orphan receptors, the family C GPCRs are dimeric receptors characterized by a large extracellular Venus flytrap domain which bind the endogenous agonists. Except from the $GABA_{B1-2}$ and T1R2–3 receptor, all receptors are either activated or positively modulated by amino acids. In this review, we outline mutational, biophysical and structural studies which have elucidated the interaction of the amino acids with the Venus flytrap domains, molecular mechanisms of receptor selectivity and the initial steps in receptor activation.
British Journal of Pharmacology (2009) **156**, 869–884; doi:10.1111/j.1476-5381.2008.00078.x; published online 4 March 2009

Keywords: G-protein-coupled receptors; family C; metabotropic glutamate receptors; calcium-sensing receptor; $GABA_B$ receptor; T1R1 taste receptor; GPRC6A receptor; amino acid sensing; mutations

Abbreviations: 7TM, seven transmembrane; CaR, calcium-sensing receptor; CRD, cysteine-rich domain; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; FRET, fluorescence resonance energy transfer; $GABA_B$, γ-aminobutyric acid type B; GPCR, G-protein-coupled receptor; GPRC6A, G-protein-coupled receptor family C, group 6, subtype A; IMP, 5′-inosine monophospate, L-CCG-I, (2*S*,1′S,2′S)-2-(carboxycyclopropyl)glycine; L-Glu, (*S*)-glutamic acid; mGlu, metabotropic glutamate; (*S*)-MCPG, (*S*)-(α)-methyl-4-carboxyphenylglycine; T1R, taste1 receptor; VFT, Venus flytrap

Introduction

The superfamily of G-protein-coupled receptors (GPCRs) contains seven transmembrane (7TM) segments and constitute a large superfamily of cell-surface proteins that are activated by a broad range of ligands (Pierce *et al.*, 2002) and are known to be implicated in many important physiological processes making them targets for approximately 40% of marketed drugs and >60% of drugs in development (Lundstrom, 2005). In the human genome, about 800 genes encode GPCRs of which 400 are olfactory receptors (Bjarnadóttir *et al.*, 2006). The non-olfactory receptors have been classified into three families (A, B and C) based on phylogenetic analysis of the 7TM domain (Kolakowski, 1994). Family C of human GPCRs contains 22 receptor subtypes including eight metabotropic glutamate (mGlu) receptors, the calcium-sensing receptor (CaR), two γ-aminobutyric acid type B ($GABA_B$) receptors, three taste1 receptors (T1R1–3), the G-protein-coupled receptor family C, group 6, subtype A (GPRC6A) and seven orphan receptors (Bjarnadóttir *et al.*, 2005; Bräuner-Osborne *et al.*, 2007)[1]. As seen in Figure 1A, the receptors cluster into four groups containing (i) the mGlu receptors; (ii) CaR, GPRC6A and T1Rs; (iii) the $GABA_B$ receptors including three orphan receptors; and (iv) a group of four RAIG1-like orphan receptors. The mGlu receptors further cluster into three subgroups (termed Group I–III) which correlates with their signal transduction pathway and orthostatic ligand pharmacology (Bräuner-Osborne *et al.*, 2007).

In the present review, we will focus on the structure, function and molecular pharmacology of the family C receptors with particular focus on the orthosteric binding pocket of the amino acid-binding subtypes. For the topic of allosteric modulation and the physiological and therapeutical roles of family C receptors, the reader is referred to recent reviews in the field (Bräuner-Osborne *et al.*, 2007; Gasparini and

Correspondence: Professor H Bräuner-Osborne, Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark. E-mail: hbo@molpharm.net
[1]The receptor nomenclature conforms to the most recent edition of the Guide to Receptors and Channels (Alexander *et al.*, 2008).
Received 4 September 2008; revised 23 October 2008; accepted 6 November 2008

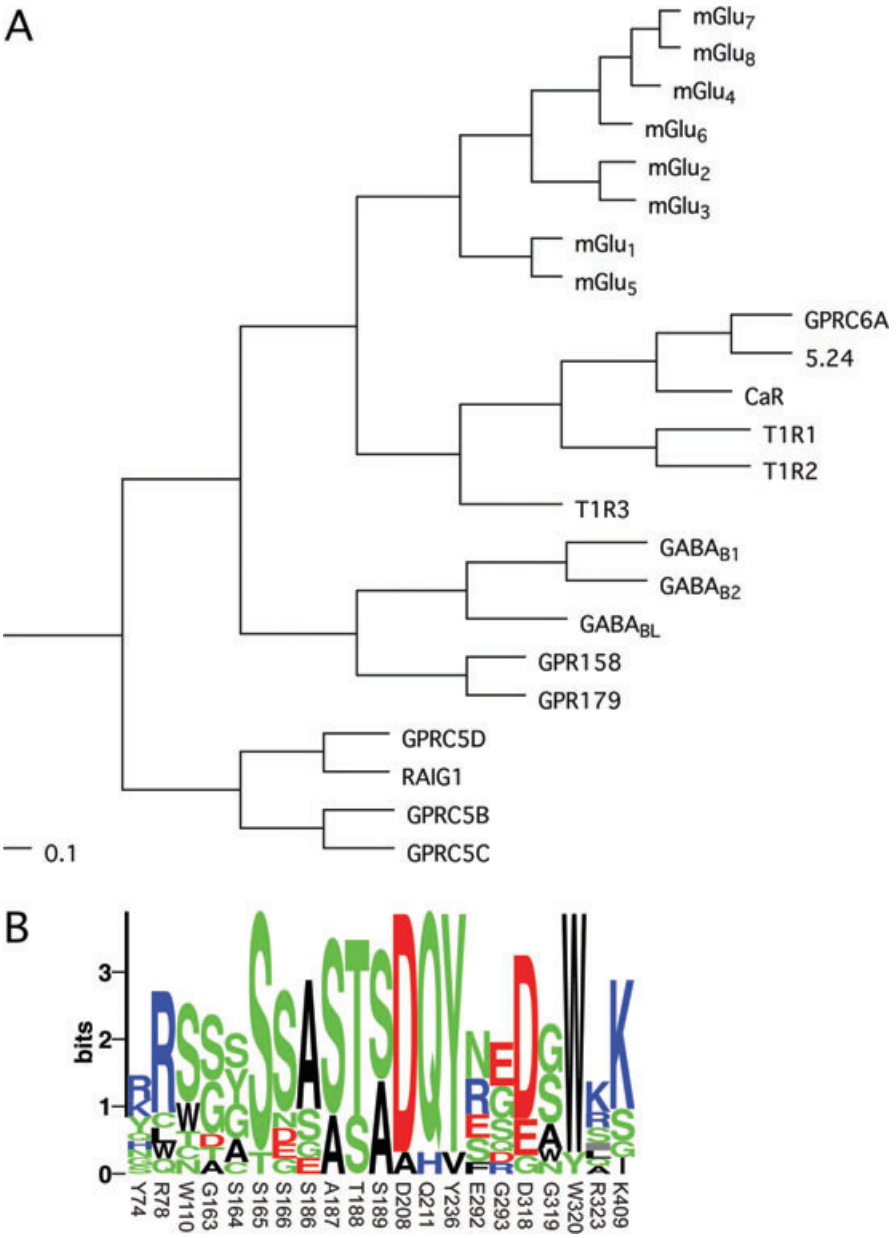

Figure 1 (A) Phylogenetic analysis of family C GPCRs based on their seven transmembrane domains. A multiple sequence alignment of the predicted seven transmembrane domains was generated using the program ClustalX 2.0.9, and the shown phylograms were generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and viewed with the program TreeviewX 0.5.0. The scale bars are a function of amino acid substitutions based on the Gonnet series substitution matrix. (B) Sequence logo of binding pocket residues in family C receptors, displaying the degree of conservation of each amino acid. Residues submitted are identical to those listed in Table 1 and numbers refer to the residue numbers in $mGlu_1$. The height of each symbol is proportional to its frequency and colour coded according to polarity. The logo was generated using the server at the Center for Biological Sequence Analysis (http://www.cbs.dtu.dk/~gorodkin/appl/plogo.html) (Schneider and Stephens, 1990; Gorodkin *et al.*, 1997). CaR, calcium-sensing receptor; $GABA_B$, γ-aminobutyric acid type B; GPCR, G-protein-coupled receptor; GPRC6A, G-protein coupled receptor family C, group 6, subtype A; mGlu, metabotropic glutamate; T1R, taste1 receptor.

Spooren, 2007; Jensen and Bräuner-Osborne, 2007; Pin and Prézeau, 2007; Trivedi *et al.*, 2008).

Structure and function of family C GPCRs

Apart from the orphan receptors, all family C GPCRs have a very large extracellular Venus flytrap (VFT) domain which contains a dimerization interface and an orthosteric binding site for the endogenous agonist (Kunishima *et al.*, 2000; Tsuchiya *et al.*, 2002; Muto *et al.*, 2007). Apart from the orphan and $GABA_B$ receptors, all family C receptors also contain a cysteine-rich domain (CRD), of unknown function, which links the VFT and 7TM domains (Figure 2). Interestingly, none of the orphan family C receptors contain a VFT domain, harbouring the orthosteric binding site, which has

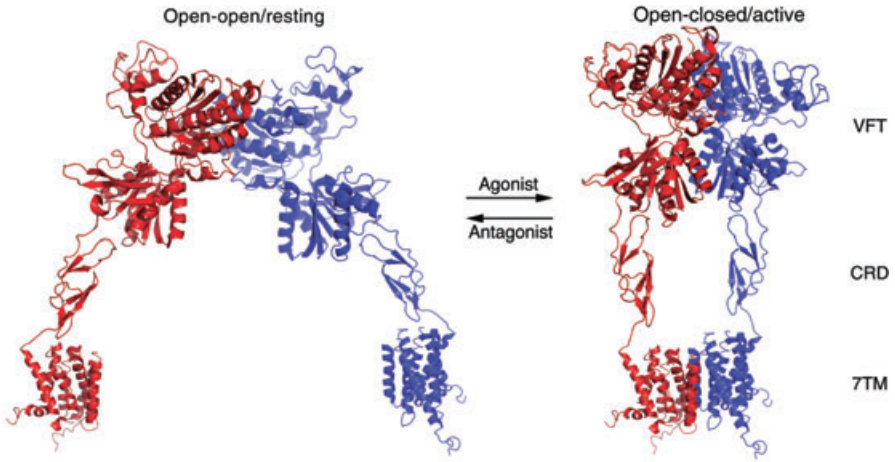

Figure 2 Model of a dimeric family C GPCR in its open–open/resting (left) and open–closed/active (right) conformations. The two conformations are in equillibrium with each other and additional conformations (not shown). Agonists and antagonists will shift the equillibrium towards the active or resting conformation respectively. The localizations of the Venus flytrap (VFT) domain, cysteine-rich domain (CRD) and seven transmembrane domain (7TM) are indicated. The models were constructed with the program MacPyMol using coordinates from PDB files 1EWT ($mGlu_1$ open–open/resting VFT), 1EWK ($mGlu_1$ open–closed/active VFT), 2E4U ($mGlu_3$ CRR) and 2R4S (β_2-adrenergic receptor 7TM). GPCR, G-protein-coupled receptor; mGlu, metabotropic glutamate.

made ligand predictions based on modelling impossible. Their endogenous ligands, if any, thus remain to be discovered (Cheng and Lotan, 1998; Bräuner-Osborne and Krogsgaard-Larsen, 2000; Robbins *et al.*, 2000; Bräuner-Osborne *et al.*, 2001; Calver *et al.*, 2003). It is also interesting to note that the three orphan receptors, $GABA_{BL}$, GPR158 and GPR179, clustering with the $GABA_B$ receptors, contain very long C-terminal domains of unknown function.

Receptor structure

The structure of the extracellular domain(s) of $mGlu_1$, $mGlu_3$ and $mGlu_7$ has been solved by X-ray crystallography, which has shown that the VFT domain consists of two distinct globular domains (termed LB1 and 2), arranged as a central ß-sheet flanked on both sides by α-helices, connected by a hinge region and separated by a cleft (Figure 2) (Kunishima *et al.*, 2000; Tsuchiya *et al.*, 2002; Muto *et al.*, 2007). The CRD consists of three ß-sheets each composed of two antiparallel ß-strands. The CRD contains nine conserved cysteines which form four intradomain disulfide bridges and a disulfide bridge to the VFT domain (Rondard *et al.*, 2006; Muto *et al.*, 2007). All nine cysteines have been shown to be imperative for receptor expression and/or activation and it is thus evident that the rigid structure of the CRD and its rigid connection to the VFT domain is important for family C receptor function (Fan *et al.*, 1998; Rondard *et al.*, 2006). The structure of a 7TM domain of a family C receptor has yet to be elucidated, but biochemical evidence point to a similar topology as the recently crystallized β_2-adrenergic family A receptor (Bhave *et al.*, 2003; Rasmussen *et al.*, 2007).

The family C GPCRs exist as constitutive dimeric receptor complexes in the cell membrane (Figure 2). Whereas the $GABA_B$ and taste1 receptors exist as heterodimeric receptors composed of two different subunits, CaR and the mGlu receptors form homodimeric complexes via several covalent and non-covalent interactions between the two subunits (Pace *et al.*, 1999; Kunishima *et al.*, 2000; Tsuji *et al.*, 2000; Romano *et al.*, 2001; Zhang *et al.*, 2001; Nomura *et al.*, 2008). The conserved inter-receptor covalent disulfide bridge (e.g. Cys140 in $mGlu_1$) is located in a loop in the VFT – a loop which has been shown by a random saturation mutagenesis study to be involved in keeping the receptor in its inactive conformation (Jensen *et al.*, 2000). In addition, the loop is a hotspot for naturally occurring activating mutations in CaR causing autosomal dominant hypocalcaemia (Hu and Spiegel, 2007). The non-covalent dimer interactions occur via a non-polar interface in LB1, whose integrity is also important for proper receptor function as mutations or introduction of an N-glycan in the interface disrupt function (Tsuji *et al.*, 2000; Rondard *et al.*, 2008).

Activation mechanism

The orthosteric binding site is located in the cleft between LB domain 1 and 2. X-ray crystallography of the $mGlu_1$ receptor has revealed that the initial event in receptor activation is closing of at least one of the VFT domains around the agonist leading to a 70° twist in the dimer interface and thus contraction of the CRD and 7TM domains (termed the 'open–closed/active conformation', Figure 2) (Kunishima *et al.*, 2000). So far, crystals of the closed–closed/active conformation has only been obtained with $mGlu_1$ in the presence of Gd^{3+} which binds to acidic residues between the LB2 domains of the dimer (Tsuchiya *et al.*, 2002; Abe *et al.*, 2003). Recently, crystals of a closed–closed/resting $mGlu_3$ VFT domain was reported (Muto *et al.*, 2007) casting doubt on the dimer interface twisting as the main mechanism of receptor activation. However, several lines of biochemical evidence support a role of the dimer interface twist as necessary for receptor activation such as an engineered antagonistic zinc-site in the interface of the two LB2 domains (in the Gd^{3+}-binding site), which prevent activation when Zn^{2+} is bound (Jensen *et al.*, 2001b), and an engineered N-glycan in the LB2 domain, which

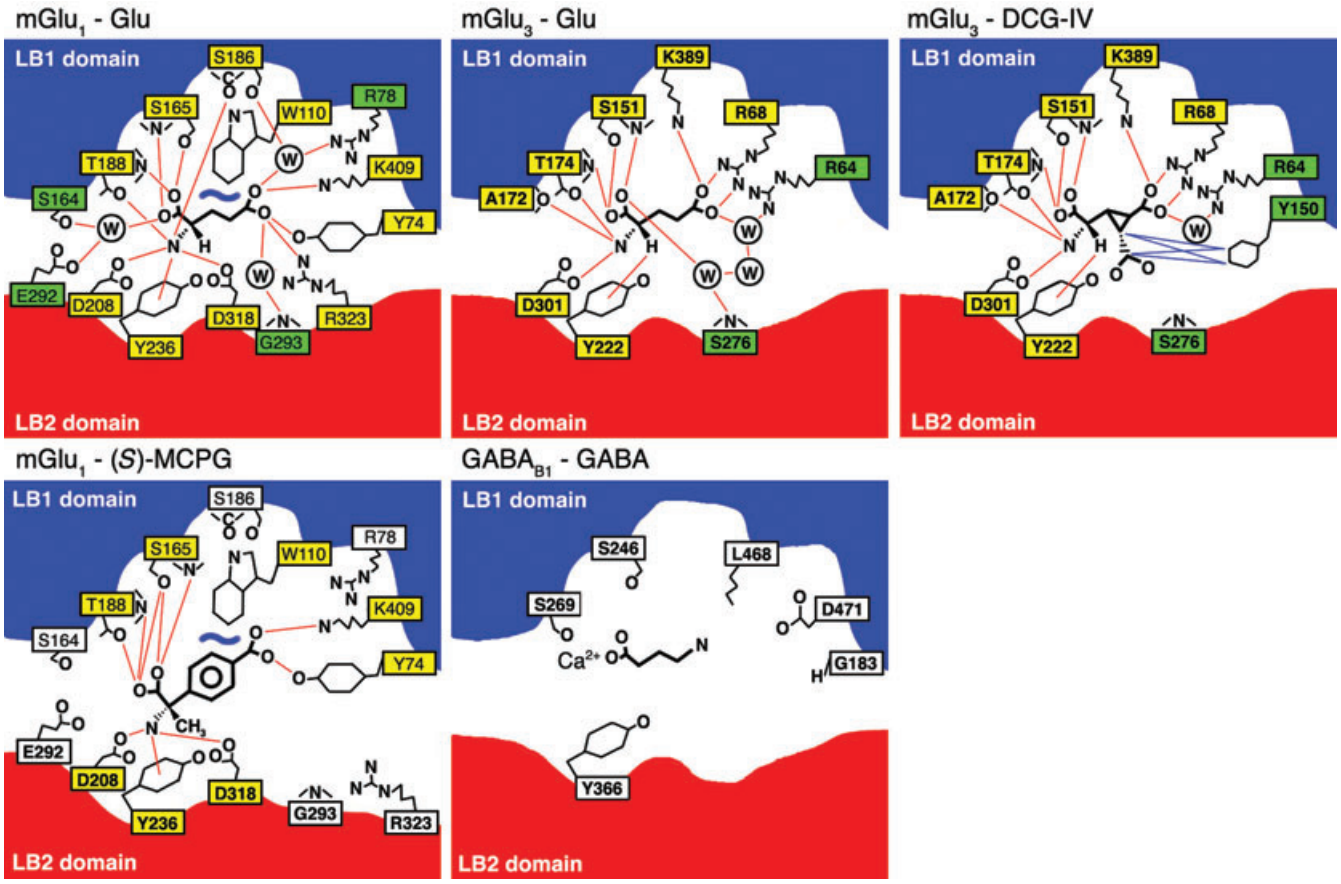

Figure 3 The orthosteric binding site in mGlu and $GABA_B$ receptors. Schematic drawings of the binding of agonists (Glu, DCG-IV or GABA) or antagonist [(*S*)-MCPG] to $mGlu_1$, $mGlu_3$ or $GABA_{B1}$. The $mGlu_1$ and $mGlu_3$ drawings are based on X-ray crystallographic structures (Kunishima *et al.*, 2000; Tsuchiya *et al.*, 2002; Muto *et al.*, 2007) whereas the $GABA_B$ drawing is based on molecular modelling and mutational data (Galvez *et al.*, 1999; 2000a; Jensen *et al.*, 2001a). Red and blue lines indicates hydrogen-bonding and van der Wahls contacts respectively. Yellow and green filled boxes indicates direct or indirect (via water) contacts respectively. Redrawn from the listed references. DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; $GABA_B$, γ-aminobutyric acid type B; mGlu, metabotropic glutamate; (*S*)-MCPG, (*S*)-(α)-methyl-4-carboxyphenylglycine.

prevent activation but not dimerization of the $GABA_B$ heterodimer (Rondard *et al.*, 2008). Several biochemical studies, using fluorescence resonance energy transfer (FRET) (Tateyama *et al.*, 2004) or rescue of activity in dimers consisting of inactivated monomers which each have been made non-functional due to mutations in either the VFT or 7TM domains (Bai *et al.*, 1999; Brock *et al.*, 2007), have also demonstrated that rearrangement of the 7TM domains are required for receptor activation. However, it remains unclear whether the rearrangement is a contraction of the 7TM domains as shown in Figure 2 or a more subtle reorientation of the 7TM domains/helices.

The crystallography studies have shown (*S*)-glutamic acid (L-Glu) binding to both open and closed VFT domains, raising the question whether closure of the VFT domain(s) is indeed the initial event in receptor activation. However, several lines of studies have pointed to this mechanism-of-activation. First of all, the X-ray structure of a $mGlu_1$ VFT with the antagonist (*S*)-(α)-methyl-4-carboxyphenylglycine [(*S*)-MCPG] shows that the antagonist serves as a wedge in the binding pocket preventing closure of the VFT (Figure 3) (Tsuchiya *et al.*, 2002). Second, this study was elaborated by an elegant study in which mutations enlarging the VFT-binding pocket of $mGlu_8$ converted the antagonists (1*R*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid and (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid into agonists (due to the additional space permitting VFT closure) (Bessis *et al.*, 2002). Third, introduction of cysteines in the LB1 and LB2 domains have created constitutively activated mutant $GABA_B$ receptors that presumably are locked in the closed VFT conformation by a disulfide bridge (Kniazeff *et al.*, 2004b). However, it remains debated whether closing of one (Kniazeff *et al.*, 2004a) or both (Kammermeier and Yun, 2005) VFT domains in a dimer is required for activation. Recently, X-ray crystallography and mutational studies have also shown that the low potency of L-Glu on $mGlu_7$ most likely is caused by steric hindrance among the residues surrounding the orthosteric binding pocket, thus preventing establishment of a stable, fully closed VFT domain (Rosemond *et al.*, 2004; Muto *et al.*, 2007).

Collectively, the structural and molecular pharmacology evidence points towards an activation model in which the VFT is in equilibrium between an open and closed form, and a resting and active/twisted conformation. Agonists will shift the equilibrium towards the closed VFT conformation, which

in turn will shift the interface twist equilibrium towards the active conformation. Conversely, antagonists will shift the equilibrium towards the open VFT conformation, which in turn will shift the interface twist equilibrium towards the resting conformation (Figure 2). The CRD domain acts as a rigid lever to transfer the energy of the conformational change in the VFT domains to the 7TM domains, but the exact mechanism of activation of the 7TM domains remains to be fully elucidated.

The orthosteric binding site in the VFT domain

An alignment of the amino acids residues in close (6 Å) proximity to L-Glu in the closed $mGlu_1$ VFT with the other amino acid-binding family C receptors is shown in Table 1. From this alignment (Table 1) and the X-ray crystal structures of the closed $mGlu_1$ and $mGlu_3$ L-Glu-bound VFTs (Figure 3), it is clear that particularly five residues (S165, T188, D208, Y236 and D318 in $mGlu_1$) are vital for binding of the α-amino acid moiety and that two basic residues (R78 and K409 in $mGlu_1$) are vital for binding of the distal carboxylic acid of L-Glu. The former residues have also been identified as an important motif of α-amino acid recognition in a large database mining study (Acher and Bertrand, 2005), which also identified three additional residues (R203, Q211 and G237 in $mGlu_1$) as part of the motif, which however do not make direct contacts with the amino acid (Figure 3). Mutational, modelling and phylogenetic studies have also suggested the existence of a Ca^{2+}-binding site adjacent to the amino acid-binding site in the VFT domain, which will be discussed in further detail below.

Molecular pharmacology of mGlu receptors

Receptor subtypes

As previously noted, the mGlu receptors have been divided into three groups (Figure 1). Receptors within a group show more than 60% sequence identity whereas there is 40–50% sequence identity between the groups. The grouping also coincides with the signal transduction pathways used by the receptors. Thus, Group I receptors stimulate phospholipase C causing an increase in intracellular inositol phosphates and Ca^{2+} levels whereas both Group II and III inhibit adenylate cyclase causing a decrease in intracellular cyclic AMP levels (Bräuner-Osborne *et al.*, 2000). Finally, receptors within the three groups also share pharmacological properties. Thus, as will be explained in greater detail in later sections, selective agonists and antagonists in most cases affect all receptors within a group. However, recently a number of ligands, in particular allosteric modulators, with specific activity for just one receptor subtype have been discovered. The extensive pharmacology of orthosteric and allosteric ligands has recently been reviewed in great detail (Schoepp *et al.*, 1999; Bräuner-Osborne *et al.*, 2000; 2007; Madsen *et al.*, 2005; Ritzén *et al.*, 2005; Gasparini and Spooren, 2007) and will thus not be reviewed in detail here.

Mechanism for agonist selectivity

The difficulties in designing subtype-specific orthosteric ligands are not surprising when taking a closer look at the

Table 1 Orthosteric binding site homology of family C receptor subtypes[a]

$mGlu_1$	***Y74***	***R78***	W110	G163	***S164***	***S165***	S166	***S186***	A187	***T188***	S189	***D208***	Q211	***Y236***	***E292***	***G293***	***D318***	G319	W320	***R323***	***K409***
$mGlu_5$	Y64	R68	W100	G149	S150	S151	S152	S172	A173	T174	S175	D194	Q197	Y222	E278	G279	D304	G305	W306	R309	K395
$mGlu_2$	R57	R61	S93	S143	Y144	S145	D146	A166	S167	T168	S169	D188	Q191	Y226	R271	S272	D295	G296	W297	L300	K377
$mGlu_3$	***R64***	***R68***	S100	S149	Y150	**S151**	S152	**A172**	S173	**T174**	S175	D194	Q197	**Y222**	R277	***S278***	**D301**	G303	W303	Q306	***K389***
$mGlu_4$	K74	R78	S110	S157	G158	S159	S160	A180	S181	T182	A183	D202	Q205	Y230	N286	E287	D312	S313	W314	K317	K405
$mGlu_6$	Q58	R62	S94	S146	A147	S148	S149	A169	S170	T171	A172	D191	Q194	Y219	N275	E276	D301	S302	W303	K306	K394
$mGlu_7$	N74	R78	S110	S157	G158	S159	S160	A180	S181	T182	A183	D202	Q205	Y230	N288	D289	D314	S315	W316	K319	K407
$mGlu_8$	K71	R75	S107	A154	A155	S156	S160	A177	S178	T179	A180	D199	Q202	Y227	N283	E284	D309	S310	W311	K314	K401
CaR	R66	W70	N102	T145	G146	S147	G148	A168	S169	S170	S171	D190	Q193	Y218	S272	G273	E297	A298	W299	S302	I416
GPRC6A	S69	Q73	T104	G147	Y148	S149	E150	E170	S171	T172	A173	D192	Q195	Y220	R279	Q280	D303	N304	W305	A308	L411
T1R1	H71	L75	S107	D147	S148	T149	N150	A170	A171	S172	S173	D192	Q195	Y220	S276	R277	E301	A302	W303	S306	S385
$GABA_{B1a}$	G183	C187	C220	G244	C245	S246	S247	G267	S268	S269	S270	A289	H292	V317	F365	E367	G393	W394	Y395	–	G461

[a]List of residues within 6 Å of L-Glu bound to $mGlu_1$ (Madsen *et al.*, 2005) based on previously published protein sequence alignments (Bräuner-Osborne *et al.*, 1999; Galvez *et al.*, 1999; Wellendorph and Bräuner-Osborne, 2004). Residues in bold are known to interact with L-Glu either directly or indirectly via water molecules in $mGlu_1$ and $mGlu_3$ respectively (Kunishima *et al.*, 2000; Muto *et al.*, 2007). Underlined and italic residues interact with the α-amino acid or distal carboxylic acid moiety of L-Glu respectively (Kunishima *et al.*, 2000; Muto *et al.*, 2007). Highly conserved residues involved in binding of the α-amino acid moiety or the distal carboxylic acid moiety of L-Glu are shown in red and green respectively.
CaR, calcium-sensing receptor; $GABA_B$, γ-aminobutyric acid type B; GPRC6A, G-protein-coupled receptor family C, group 6, subtype A; mGlu, metabotropic glutamate; T1R, taste1 receptor.

orthosteric binding pocket. The 21 amino acid residues within 6 Å of L-Glu bound to the closed VFT of $mGlu_1$ are given in Table 1 and the degree of conservation is shown in Figure 1B. Nine of these residues are identical in all eight mGlu receptors, which mainly participate in the previously discussed motifs recognizing the α-amino acid moiety and distal carboxylic acid of L-Glu. All 21 amino acid residues are identical between $mGlu_1$ and the other Group I receptor $mGlu_5$, only two of the 21 amino acid residues differ between the Group II receptors $mGlu_2$ and $mGlu_3$, and only four of the 21 amino acid residues differ between the Group III receptors $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ (Table 1). A large number of mutagenesis studies have investigated the importance of individual residues in the binding pocket (Table 2). Not surprisingly, these studies have pointed out the five highly conserved residues binding the α-amino acid moiety (S165, T188, D208, Y236 and D318 in $mGlu_1$) and the two highly conserved basic residues binding the distal carboxylic acid of L-Glu (R78 and K409 in $mGlu_1$) as being of particular importance for agonist binding (Tables 1 and 2, Figure 3). Several studies have addressed the basis for Group selectivity. Based on modelling and ligand docking, we suggested that differences in the shape and electronic environment of the binding pocket of $mGlu_1$ and $mGlu_4$ as the basis for selectivity of ibotenic acid for the former subtype (Hermit *et al.*, 2004). Subsequent mutational studies showed that binding affinities of the Group I selective agonists ibotenic acid and quisqualic acid were dramatically increased at $mGlu_4$ when three residues in the receptor concomitantly were mutated to the corresponding residues in $mGlu_1$ (K74Y, E287G and K317R). The individual mutations had only minor effects which led to the conclusion that the subtype selectivity arises from a synergy of contributions and a complex interplay of residues shaping the binding pocket, rather than being attributable to a single specific ligand–receptor interaction. The recent X-ray crystallography structure of the $mGlu_3$ VFT complexed with L-Glu or several Group II selective agonists has revealed a similar complex interplay of several residues providing subtype selectivity. Thus, it had previously been shown that mutation of Y150A, Y222A or R277A dramatically reduced the potency of (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) but not (2*S*,1′S,2′S)-2-(carboxycyclopropyl)glycine at $mGlu_3$ (Table 2), which led the authors to suggest that the extra carboxylic acid moiety of DCG-IV at position C3′ points towards these three residues (Yao *et al.*, 2003). Y222 is identical in all eight mGlu receptors and can thus not contribute to the Group II selectivity of DCG-IV, whereas Y150 and R277 are indeed only present in Group II (Table 1). However, the recent X-ray crystallography structure of the $mGlu_3$ VFT complexed with DCG-IV shows that only Y150 interacts directly with the agonist via van der Wahls contacts (Figure 3) (Muto *et al.*, 2007). Based on this observation, it was suggested that the R227 residue played a role in supporting the rigid ligand-binding pocket, thus participating in ligand selectivity via an indirect mechanism rather than direct interaction with the ligand (Muto *et al.*, 2007).

It is interesting to note the difference in the positions of the water molecules in the L-Glu-bound $mGlu_1$ and $mGlu_3$ VFTs (Figure 3). Likewise, it is interesting to note that $mGlu_3$ accommodates the bulkier agonist DCG-IV by replacement of two water molecules by the additional carboxylic acid group rather than by repositioning of the amino acid side chains in the binding pocket (Figure 3). The higher potency of DCG-IV compared with L-Glu at $mGlu_3$ is thus obtained by the lower cost of binding energy of the former ligand in terms of solvent entropy (Muto *et al.*, 2007). Given the different positioning of the water molecules in $mGlu_1$, DCG-IV cannot obtain a similar energy advantage at this subtype, which is one possible explanation for the Group II selectivity of the ligand.

Collectively, these studies show that several different molecular mechanisms play a role in subtype/group selectivity which makes structure-based ligand design very challenging.

Molecular pharmacology of the $GABA_B$ receptor

The $GABA_B$ receptor was first identified in the early eighties on the basis of pharmacological responses to the agonist baclofen and insensitivity to the $GABA_A$ antagonist bicuculline (Bowery *et al.*, 1980; Hill and Bowery, 1981) but resisted cloning until the late nineties (Kaupmann *et al.*, 1997). The $GABA_B$ receptor is coupled to Gαi proteins and activation causes a decrease in Ca^{2+} and an increase in K^+ membrane conductance and inhibition of cyclic adenosine monophosphate (cAMP) formation (Bowery *et al.*, 2002; Bettler *et al.*, 2004). The $GABA_B$ receptor is a heterodimer consisting of the $GABA_{B1}$ and $GABA_{B2}$ subunits (Jones *et al.*, 1998; Kaupmann *et al.*, 1998; White *et al.*, 1998; Kuner *et al.*, 1999) of which the former contains the orthosteric GABA-binding site and the latter appears to be primarily involved in G-protein coupling (Pin *et al.*, 2004). Interestingly, the $GABA_{B2}$ subunit does contain a VFT domain but phylogenetic analysis has shown that it is unlikely to contain a ligand-binding site (Kniazeff *et al.*, 2002). The heterodimerization is controlled by an RSRR motif in the C-terminal of $GABA_{B1}$ which traps the subunit in the endoplasmatic reticulum unless it is masked by a coiled-coil interaction with the C-terminal of $GABA_{B2}$ enabling the trafficking of both subunits to the plasma membrane (Pin *et al.*, 2004). Initial cloning efforts revealed two major amino-terminal isoforms, $GABA_{B1(a)}$ and $GABA_{B1(b)}$. The former contains two amino-terminal sushi-repeats, which are protein–protein interaction motifs that are expected to serve as an extracellular targeting signal that dictates subcellular localization (Kaupmann *et al.*, 1997).

Extensive mutational analysis of the $GABA_{B1}$ VFT has revealed surprisingly few residues with substantial effects on GABA or baclofen potency (Table 3). Mutations of S246 and D471 lead to significantly lower potencies of both agonists while mutation of Y366 has a moderate effect (Galvez *et al.*, 1999; 2000a; Jensen *et al.*, 2001a). Ca^{2+} allosterically modulates the potency of GABA but not baclofen. Presumably Ca^{2+} interacts with S269 and stabilizes the active closed conformation of the VFT (Galvez *et al.*, 2000b; Jensen *et al.*, 2001a). Together with molecular modelling, these results have led to a model where the carboxylic acid of GABA binds to S246 and S269 (via Ca^{2+}) and the amine of GABA binds to D471 (Figure 3) (Galvez *et al.*, 2000a; Costantino *et al.*, 2001).

Despite plenty of *in vitro* and *in vivo* evidence of distinct pharmacological $GABA_B$ receptor subtypes, the $GABA_{B1(a)}$ and

Table 2 The effect on agonist potency of engineered mutations in or near the ligand-binding pocket of metabotropic glutamate receptors

Residue	*Potency*[a]	*Agonist*[b] *(assay*[c]*)*	*References*
$mGlu_1$			
Y74E	↓↓↓	Glu (IP), Quis (B)	(Sato *et al.*, 2003)
R78A/L/E	↓↓(↓)	Glu (IP), Quis (IP, B)	(Jensen *et al.*, 2000; Sato *et al.*, 2003)
S164A	=	Glu (IP, B), Quis (IP, B)	(O'Hara *et al.*, 1993; Sato *et al.*, 2003)
S165A	↓↓(↓)	Glu (IP), Quis (IP, B)	(O'Hara *et al.*, 1993; Sato *et al.*, 2003)
S166A	=	Glu (EP)	(Kubo *et al.*, 1998)
S186A	=	Glu (IP), Quis (IP, B)	(O'Hara *et al.*, 1993; Sato *et al.*, 2003)
T188A	↓↓↓	Glu (IP), Quis (IP,B)	(O'Hara *et al.*, 1993; Sato *et al.*, 2003)
D208A	↓↓↓	Glu (IP), Quis (B)	(Sato *et al.*, 2003)
Y236A	↓↓↓	Glu (IP), Quis (B)	(Sato *et al.*, 2003)
E292A	↓↓↓	Quis (B)	(Sato *et al.*, 2003)
G293A	↓↓↓	Glu (IP), Quis (B)	(Sato *et al.*, 2003)
D318A	↓↓↓	Glu (IP), Quis (B)	(Sato *et al.*, 2003)
R323A	=	Glu/Quis (B)	(Sato *et al.*, 2003)
K409A	=	Glu/Quis (B)	(Sato *et al.*, 2003)
$mGlu_2$			
R57A/Y	↓↓(↓)	Glu (EP), LY354740 (EP, B)	(Malherbe *et al.*, 2001)
Y144A/S/G/F	= (↓)	Glu (G, EP), LY354740 (G, EP, B)	(Malherbe *et al.*, 2001)
S145A	↓↓↓	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
D146S	=	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
S148A	>↓	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
S164A	=	LY354740 (B)	(Malherbe *et al.*, 2001)
A166S	=	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
S167A	=	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
T168A	↓(↓↓)	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
S169A	=	Glu (G), LY354740 (G, B)	(Malherbe *et al.*, 2001)
R183A	↓	Glu (EP), LY354740 (EP, B)	(Malherbe *et al.*, 2001)
D188A	↓↓↓	DCG-IV (B)	(Yao *et al.*, 2003)
Y216A/F	↓↓↓	LY354740 (B)	(Malherbe *et al.*, 2001)
R271A	=	LY354740 (B)	(Malherbe *et al.*, 2001)
	↓↓	DCG-IV (B)	(Yao *et al.*, 2003)
D295A/R	↓↓↓	Glu (EP), LY354740 (EP, B)	(Malherbe *et al.*, 2001)
L300A	↓	DCG-IV (B)	(Yao *et al.*, 2003)
K377A	↓↓↓	DCG-IV (B)	(Yao *et al.*, 2003)
$mGlu_3$			
R64A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
R68A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
Y150A	↓↓(↓)	DCG-IV (C, B)	(Yao *et al.*, 2003)
	=	L-CCG-I (C)	(Yao *et al.*, 2003)
S151A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
S152D/H	=	LY354740 (B)	(Malherbe *et al.*, 2001)
T174A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
D194A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
Y222A	↓↓↓	DCG-IV (C, B)	(Yao *et al.*, 2003)
	=	L-CCG-I (C)	(Yao *et al.*, 2003)
R277A	↓↓(↓)	DCG-IV (C, B)	(Yao *et al.*, 2003)
	=	L-CCG-I (C)	(Yao *et al.*, 2003)
D301A	↓↓↓	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
Q306A	↓↓↓	DCG-IV (B)	(Yao *et al.*, 2003)
K389A	↓↓(↓)	DCG-IV (C, B), L-CCG-I (C)	(Yao *et al.*, 2003)
$mGlu_4$			
K74A/Y/N/Q	=	L-AP4 (B)	(Rosemond *et al.*, 2002; Hermit *et al.*, 2004)
K74Y	↓↓↓	L-AP4 (B)	(Rosemond *et al.*, 2002)
H77Q	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
R78A	↓↓↓	L-AP4 (B)	(Hampson *et al.*, 1999)
S157A	=	Glu (C), L-AP4 (B)	(Hampson *et al.*, 1999; Frauli *et al.*, 2007)
G158A	=	Glu (C), L-AP4 (B)	(Hampson *et al.*, 1999; Frauli *et al.*, 2007)
S159A	↓↓↓	L-AP4 (B)	(Hampson *et al.*, 1999)
S160A	=	L-AP4 (B)	(Hampson *et al.*, 1999)
S181A	=	L-AP4 (B)	(Hampson *et al.*, 1999)
T182A	↓↓↓	L-AP4 (B)	(Hampson *et al.*, 1999)
D202A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
Y230A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
R258A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
N286A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
E287A	↓(↓↓)	L-AP4 (B)	(Rosemond *et al.*, 2002; Hermit *et al.*, 2004)
D312A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
S313G	=	L-AP4 (B)	(Hermit *et al.*, 2004)
K317A/R	=	L-AP4 (B)	(Rosemond *et al.*, 2002; Hermit *et al.*, 2004)
K405A	↓↓↓	L-AP4 (B)	(Rosemond *et al.*, 2002)
$mGlu_7$			
N74K	↑	L-AP4 (B)	(Rosemond *et al.*, 2004)
$mGlu_8$			
K71A	=	L-AP4 (B)	(Rosemond *et al.*, 2002)
K71Y	↓↓↓	L-AP4 (B)	(Rosemond *et al.*, 2002)
R75A	↓↓↓	L-AP4 (B)	(Rosemond *et al.*, 2002)
A154S	=	Glu (C)	(Frauli *et al.*, 2007)
A155S	=	Glu (C)	(Frauli *et al.*, 2007)
Y227A/F	= (↓)	Glu/L-AP4 (IP)	(Bessis *et al.*, 2002)
D309A	↓↓(↓)	Glu/L-AP4 (IP)	(Bessis *et al.*, 2002)
D309E	= (↓)	Glu (IP)	(Bessis *et al.*, 2002)

[a]Potency of mutant compared with wild-type receptor: ↑, 10- to 50-fold increase; =, less than 10-fold difference; ↓, 10- to 50-fold decrease or reduced binding/response; ↓↓, 50- to 500-fold decrease or weak binding/response; ↓↓↓, >500-fold decrease or no binding/response.
[b]DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; Glu, L-glutamic acid; L-AP4, L-amino-4-phosphonobutyric acid; L-CCG-I, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine; LY354740, (+)-2-aminobicyclo-[3.1.0]-hexane-2,6-dicarboxylate; Quis, L-quisqualic acid.
[c]B, binding; C, intracellular [Ca^{2+}]; EP, electrophysiology; G, [^{35}S]GTPγS binding; IP, inositol phosphate.

Table 3 The effect on agonist potency of engineered mutations in or near the ligand binding pocket of the $GABA_{B1}$ receptor

Residue[a]	*Potency*[b]	*Agonist (assay*[c]*)*	*References*
C187A/S	=	GABA (B, IP)	(Galvez *et al.*, 1999; 2000a)
Q188A	=	GABA (B)	(Galvez *et al.*, 1999)
E192A	=	GABA (B)	(Galvez *et al.*, 2000a)
S246A/P/T/N	↓↓↓	GABA/baclofen (IP, B)	(Galvez *et al.*, 1999; 2000a; Jensen *et al.*, 2001a)
S247A	= (↑)	GABA/baclofen (IP, B)	(Galvez *et al.*, 1999; 2000b)
S249A	=	GABA (B)	(Galvez *et al.*, 1999)
T250A	=	GABA (B)	(Galvez *et al.*, 1999)
S265A	=	GABA (IP)	(Galvez *et al.*, 2000a)
Y266F	=	GABA/baclofen (IP)	(Galvez *et al.*, 2000a)
S268A	=	GABA/baclofen (B, IP)	(Galvez *et al.*, 1999; 2000b)
S269A	= (↓)	GABA/baclofen (B, IP)	(Galvez *et al.*, 1999; 2000b; Jensen *et al.*, 2001a)
S270A	↓	GABA/baclofen (B, IP)	(Galvez *et al.*, 1999; 2000a)
T310A	=	GABA (B)	(Galvez *et al.*, 1999)
Q312A	=	GABA/baclofen (IP, B)	(Galvez *et al.*, 1999; 2000a)
Q313A	=	GABA (B)	(Galvez *et al.*, 1999)
T314A	=	GABA (B)	(Galvez *et al.*, 1999)
T315A	=	GABA (B)	(Galvez *et al.*, 1999)
E316A	=	GABA (B)	(Galvez *et al.*, 1999)
F365A	=	GABA/baclofen (IP, B)	(Galvez *et al.*, 2000a)
Y366A	↓(↓)	GABA/baclofen (IP, B)	(Galvez *et al.*, 2000a)
F367A	=	GABA/baclofen (IP, B)	(Galvez *et al.*, 2000a)
E458A	=	GABA (B)	(Galvez *et al.*, 1999)
E459A	=	GABA (B)	(Galvez *et al.*, 1999)
T460A	=	GABA (B)	(Galvez *et al.*, 1999)
F463A	=	GABA/baclofen (IP)	(Galvez *et al.*, 2000a)
Q464A	=	GABA (B)	(Galvez *et al.*, 1999)
E465A	= (↓)	GABA (B, IP)	(Galvez *et al.*, 2000a)
Y470A/F	=	GABA (IP)	(Galvez *et al.*, 2000a)
D471A/E	↓↓↓	GABA/baclofen (IP, B)	(Galvez *et al.*, 2000a)

[a]Numbering according to the $GABA_{B1a}$ variant.
[b]Potency of mutant compared to wild-type receptor: ↑, 10- to 50-fold increase; =, less than 10-fold difference; ↓, 10- to 50-fold decrease or reduced binding/response; ↓↓, 50- to 500-fold decrease or weak binding/response; ↓↓↓, >500-fold decrease or no binding/response.
[c]B, binding; $GABA_B$, γ-aminobutyric acid type B; IP, inositol phosphate.

$GABA_{B1(b)}$ isoforms display no differences in binding or functional experiments with native (Malitschek *et al.*, 1998) or recombinant expression (Kaupmann *et al.*, 1997; 1998; Bräuner-Osborne and Krogsgaard-Larsen, 1999). Together with the group of Bettler, we developed isoform-specific $GABA_{B1(a)}$ and $GABA_{B1(b)}$ knockout mice which have shown that the two isoforms display distinct expression patterns at the synaptic level via which the isoforms control different effector systems and physiological functions (Pérez-Garci *et al.*, 2006; Vigot *et al.*, 2006). It thus seems evident that the pharmacological subtypes arise from spatial control of expression, rather than a multitude of receptor subtypes.

Molecular pharmacology of the CaR

Orthosteric ligands

As the name implies, the CaR functions to sense Ca^{2+} and does so by responding to millimolar concentrations of Ca^{2+} present in the extracellular fluid, and hereby regulates the release of hormones important for maintaining calcium homeostasis of the organism [for reviews see (Tfelt-Hansen and Brown, 2005) and (Brown, 2007)]. In addition to Ca^{2+}, CaR is also activated by other inorganic cations in the millimolar range, including Mg^{2+}, Ba^{2+} and Sr^{2+}, as well as Gd^{3+} in the micromolar range (Brown *et al.*, 1993; Ruat *et al.*, 1996; Coulombe *et al.*, 2004). Both Ca^{2+} and Mg^{2+} exhibit cooperativity in binding, yielding Hill coefficients aroung 3–5 (Bai *et al.*, 1996; Ruat *et al.*, 1996; Bräuner-Osborne *et al.*, 1999), which from a physiological point of view allows for differentiation of the CaR response by only minute fluctuations in agonist concentrations.

Similar to other family C receptors, CaR is also sensitive to L-α-amino acids. This is not surprising given the high degree of conservation of key residues known to be important in binding of L-Glu in $mGlu_1$ (Table 1). However, whereas other family C receptors (mGlu, $GABA_B$ and GPRC6A receptors) are directly activated by amino acids and positively modulated by calcium, CaR operates in a reciprocal fashion, being directly activated by Ca^{2+} and positively modulated by amino acids. Furthermore, while mGlu receptors respond only to L-Glu (Frauli *et al.*, 2006), the group of CaR, GPRC6A and T1R1+T1R3 are less selective and respond to several classes of amino acids. In fact, each of these receptors prefer different classes of amino acids and are able to cover a wide range of amino acid selectivities in concert (Figure 4), suggested to be of physiological relevance for instance in the gut (Conigrave and Brown, 2006; Conigrave *et al.*, 2008). At CaR, the most potent are the aromatic amino acids L-Phe and L-Trp, followed by aliphatic and polar amino acids. The least potent are the branched-chain, basic and sulfur-containing amino acids (Conigrave *et al.*, 2000). Specifically for CaR, the potential physiological relevance of the positive modulation of receptor activity by amino acids has been demonstrated in the parathyroid and the intestinal/digestive system (Conigrave *et al.*,

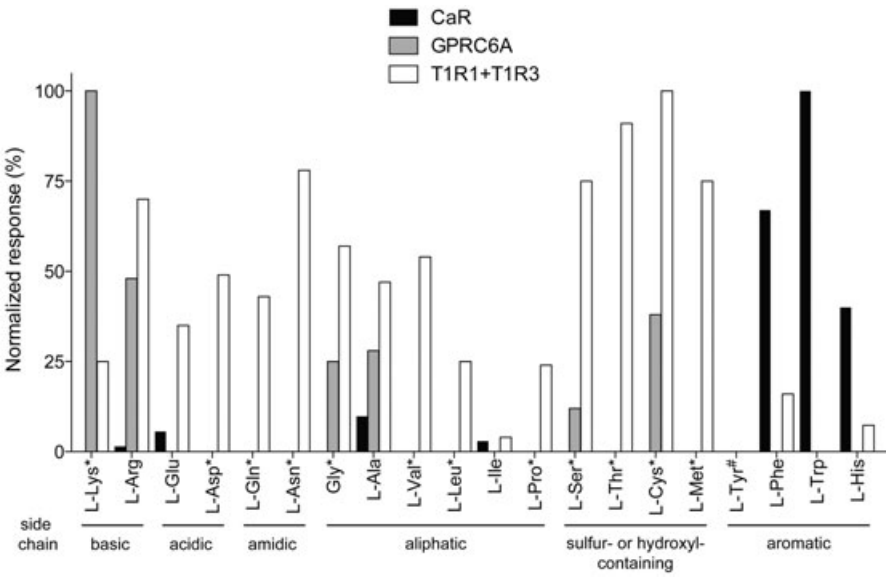

Figure 4 L-Amino acid selectivity profiles at CaR, GPRC6A and the T1R1+T1R3 heterodimer. Amino acids are grouped according to side chain charge and polarity. Data have been normalized to allow for comparison of relative amino acid preferences. The profile for CaR was generated by normalizing reported EC_{50} values from seven amino acids (measured in human parathyroid cells in the presence of 2 mmol·L^{-1} Ca^{2+}) to the response of L-Trp (set to 100%). Amino acids marked with asterices (*) were not included in the original study. The profile for GPRC6A is based on reported EC_{50} values from mouse GPRC6A measured in the presence of 1 mmol·L^{-1} Ca^{2+} and 1 mmol·L^{-1} Mg^{2+} (Christiansen *et al.*, 2007) and here normalized to the L-Lys response (set to 100%). T1R1+T1R3 data originally in the form of 'number of responsive cells' measured in the presence of 2.5 mmol·L^{-1} IMP (Nelson *et al.*, 2001) were converted to percentage normalized response by calculating the response relative to that of L-Cys (set to 100%). The symbol (#) denotes that L-Tyr was not tested at T1R1+T1R3 (due to insolubility). CaR, calcium-sensing receptor; GPRC6A, G-protein coupled receptor family C, group 6, subtype A; T1R, taste1 receptor.

2004; Busque *et al.*, 2005; Hira *et al.*, 2008), and suggested in several other tissues (Conigrave *et al.*, 2007; 2008).

Other positive modulators at CaR include polyvalent molecules (including spermine, spermidine), β-amyloid peptides, several aminoglycoside antibiotics (Ruat *et al.*, 1996; Quinn *et al.*, 1997; Ye *et al.*, 1997; Brown and MacLeod, 2001) along with increases in pH and ionic strength (Quinn *et al.*, 1998; Quinn *et al.*, 2004). Furthermore, it was recently found that the binding pocket of CaR (and the related goldfish 5.24 receptor) is in fact large enough to accommodate small peptides such as glutathione (GSH), nicely illustrated by the binding of [^{3}H]GSH to the soluble VFT domain of CaR and the ability of GSH to potentiate the Ca^{2+} response (Wang *et al.*, 2006). In addition to allosteric modulation in the VFT domain, CaR exhibits an allosteric activator site for calcimimetics and an allosteric inhibitory site for calcilytics in the 7TM domain of the receptor (Nemeth *et al.*, 1998; 2001).

The orthosteric binding site(s)

Based on studies using chimeric receptors, it has been firmly established that Ca^{2+} binding takes place in the CaR VFT domain (Bräuner-Osborne *et al.*, 1999; Hammerland *et al.*, 1999), although reports also point to the existence of separate Ca^{2+}/Gd^{3+}-binding sites in the 7TM domain (Hammerland *et al.*, 1999; Hu *et al.*, 2002; 2005; Ray and Northup, 2002). Focusing on the VFT domain-binding sites, mutagenesis studies have identified a number of residues important for Ca^{2+}-activation (Table 4); however, as no crystal structure of CaR exists and as no binding assay is available to confirm if these residues directly contact the ligand, the exact residues involved in Ca^{2+} binding have not been unequivocally demonstrated. As given in Table 4, the five residues S147, S170, D190, Y218 and E297, conserved as the basic α-amino acid recognizing motif in family C GPCRs, yield significantly decreased sensitivity to Ca^{2+} (and other cations) when mutated, suggesting that these residues are involved in cation binding (Bai *et al.*, 1996; Pearce *et al.*, 1996; Bräuner-Osborne *et al.*, 1999; Hauache *et al.*, 2000; Zhang *et al.*, 2002; Mun *et al.*, 2005). From homology models of CaR using the $mGlu_1$ crystal structure as template, these residues have as well been predicted to be part of the Ca^{2+}-binding site. In one of the more recent modelling studies, Silve *et al* predicted and validated a Ca^{2+}-binding site in the cleft of the two lobes, lying adjacent to the amino acid-binding site but being of smaller dimensions. In addition to the five participating residues already mentioned, they identified the three residues Q193, F270 and S296 to be part of the cation-binding site (Silve *et al.*, 2005). More recently, another study homology-modelled CaR and identified three distinct Ca^{2+}-binding sites, which correlates well with the experimentally determined Hill coefficient of 3–5. One of these corresponded with the one reported by Silve *et al.* Ligand binding to the two other predicted sites was validated by FRET-based methods by expressing the relevant sequences (up to 30 amino acids long) in scaffolding proteins, hereby demonstrating two novel cation-binding sites centred around E378 and E398 (Huang *et al.*, 2007).

Based on the observation that the five residues known to bind the α-amino acid moiety of L-Glu in $mGlu_1$ are conserved in CaR (Table 1), it is anticipated that an analogous amino acid-binding site exists in CaR. Mounting evidence for

Table 4 The effect of mutations in the predicted Ca^{2+} and/or L-amino acid-binding sites on the potency and efficacy of Ca^{2+} on the CaR

CaR	*Ca^{2+} sensitivity*				*Amino acid sensitivity*			
Residue no.	*Potency*	*Maximal response*	*Other observations*	*References*	*Ca^{2+} potency*	*Ca^{2+} max response*	*Other observations*	*References*
R66C/H	↓	↓	Reduced Gd^{3+} response naturally occurring inactivating mutations (FHH/NSHPT)	(Bai *et al.*, 1996; Pidasheva *et al.*, 2006)				
T145A	=	=		(Mun *et al.*, 2005)	(+)	NE	Mutation affects L/D-aa selectivity	(Mun *et al.*, 2005)
S147A	↓	↓		(Bräuner-Osborne *et al.*, 1999; Zhang *et al.*, 2002; Mun *et al.*, 2005)	+	+		(Zhang *et al.*, 2002; Mun *et al.*, 2005)
S169A	=	=		(Bräuner-Osborne *et al.*, 1999; Zhang *et al.*, 2002)	+	NE		(Zhang *et al.*, 2002)
S169T	↓=/	↓=/	Lower sensitivity in Ca^{2+} mobilization/unchanged sensitivity in ERK assays	(Lee *et al.*, 2007)	+/+	+/+	Enhanced sensitivity in both Ca^{2+} mobilization/ERK assays	(Lee *et al.*, 2007)
S170A	↓	↓		(Bräuner-Osborne *et al.*, 1999; Zhang *et al.*, 2002; Mun *et al.*, 2005)	NE	(+)		(Zhang *et al.*, 2002; Mun *et al.*, 2005)
S170T	=	=		(Mun *et al.*, 2005)	(+)	NE		(Mun *et al.*, 2005)
T145A/S170T	=	=		(Mun *et al.*, 2005)	NE	NE		(Mun *et al.*, 2005)
S171A	(↓)	↑		(Bräuner-Osborne *et al.*, 1999; Zhang *et al.*, 2002				
S169–171A	↓	↓		(Mun *et al.*, 2005)	NE	NE		(Mun *et al.*, 2005)
D190A/K	↓	↓		(Hauache *et al.*, 2000; Zhang *et al.*, 2002)	+	+		Zhang *et al.*, 2002)
Q193A	↓	=		(Silve *et al.*, 2005)				
Y218A/S/F	↓	↓		Mun *et al.*, 2005; Zhang *et al.*, 2002)	+	+		(Zhang *et al.*, 2002; Mun *et al.*, 2005)
Y218S	↓	↓	Only mild attenuation of Gd^{3+} response Naturally occurring inactivating mutation (NSHPT)	(Pearce *et al.*, 1996; Zhang *et al.*, 2002)	+	+		
E224I E228/229I	(←)	↓		(Huang *et al.*, 2007)				
F270A	↓	↓		(Silve *et al.*, 2005)				
S296A	↑	↑		(Silve *et al.*, 2005)				
E297K/I/Q	↓	↓	Reduced Gd^{3+} response naturally occurring inactivating mutation (FHH/NSHPT)	(Bai *et al.*, 1996; Hauache *et al.*, 2000; Zhang *et al.*, 2002; Mun *et al.*, 2005; Huang *et al.*, 2007)	+	+		(Zhang *et al.*, 2002; Mun *et al.*, 2005)
E297D	↑	=	Naturally occurring activating mutation (ADH)	(Silve *et al.*, 2005				
E378/379I	↑	↑		(Huang *et al.*, 2007)				
E398/399I	↓	↓		(Huang *et al.*, 2007)				

ADH: autosomal dominant hypocalcaemia; CaR, calcium-sensing receptor; ERK, extra cellular signal regulated kinase; FHH: familial hypercalciuric hypocalcaemia; NE: no effect; NSPHT: neonatal severe hyperparathyroidism.

the location of the amino acid-binding site to the VFT domain has come from chimeric receptor studies (Mun *et al.*, 2004), from the finding that L-Phe and the allosteric modulator, NPS R-467, known to bind in the 7TM domain of CaR, act synergistically and hence at distinct binding sites (Zhang *et al.*, 2002), as well as from site-directed mutageneses (collected in Table 4). It has, however, been inherently difficult to pinpoint residues specifically involved in amino acid binding and activation, as the amino acids require the presence of Ca^{2+} to work and many of the examined mutations simultaneously reduce Ca^{2+} sensitivity. Mun *et al.* identified two mutations, T145A and S170T that specifically impair amino acid-sensing while leaving Ca^{2+} sensing intact (Mun *et al.*, 2005). Others have also identified the three serines S169–171 as being important for amino acid binding (Zhang *et al.*, 2002; Lee *et al.*, 2007). Obviously, as CaR prefers aromatic amino acids and is not sensitive to L-Glu, the residues involved in binding of the distal end of L-Glu are not conserved in CaR (Table 1), exemplified by the lack of conservation of residues corresponding to the $mGlu_1$ residues Y74 and R323 to CaR (Silve *et al.*, 2005). It remains to be investigated which residues participate in binding of the aromatic moieties of L-Phe/L-Trp in the CaR VFT domain.

Molecular pharmacology of GPRC6A

GPRC6A was originally identified using bioinformatics based on sequence identity to known family C GPCRs and has subsequently been molecularly cloned from human, mouse and rat (Wellendorph and Bräuner-Osborne, 2004; Kuang *et al.*, 2005; Wellendorph *et al.*, 2005; 2007). As illustrated in the phylogenetic tree (Figure 1A), GPRC6A is most closely related to the goldfish 5.24 receptor, which based on ligand preferences for basic amino acids (Speca *et al.*, 1999; Christiansen *et al.*, 2006a) is believed to be the GPRC6A orthologue in this species. Of the human family C receptors, CaR is the closest homologue.

Orthosteric ligands

GPRC6A is stereoselectively activated by natural L-α-amino acids, preferentially basic amino acids L-Arg, L-Lys and L-ornithine (L-Orn), but also small and polar amino acids, whereas aromatic amino acids are inactive (Kuang *et al.*, 2005; Wellendorph *et al.*, 2005; 2007; Christiansen *et al.*, 2007). Furthermore, we have identified a number of synthetic derivatives of L-Arg, L-Lys and L-Orn, otherwise known to be regulators of the nitric oxide synthase and arginase isoenzymes, to be GPRC6A agonists (Christiansen *et al.*, 2006b; Hrabák, 2006). This sensitivity profile is complementary to CaR, and both GPRC6A and CaR also differ from T1R1+T1R3 with respect to amino acid preferences (Figure 4) (Conigrave and Brown, 2006; Bräuner-Osborne *et al.*, 2007). Using heterologous expression systems, we and others have found that the L-α-amino acid response of GPRC6A is augmented by divalent cations Ca^{2+} and Mg^{2+} in physiological relevant concentrations (Kuang *et al.*, 2005; Christiansen *et al.*, 2007; Wellendorph *et al.*, 2007) and one report has even demonstrated a direct activation of GPRC6A by Ca^{2+} (Pi *et al.*, 2005). GPRC6A thus bears the general characteristic of a family C receptor in having a dual responsiveness to Ca^{2+} and amino acids. To date, no antagonists have been identified at GPRC6A.

The orthosteric binding site

So far, only few studies have addressed the molecular basis of amino acid activation of GPRC6A. We have generated a homology model of the human GPRC6A with L-Lys docked into the predicted binding pocket based on the $mGlu_1$ crystal as template, and constructed under the assumption that the binding site orientation of L-Lys in GPRC6A substantially resembles that of L-Glu in the mGlu receptors (Wellendorph *et al.*, 2005) – an assumption shared by Kuang *et al.* in their model of L-Lys bound to the goldfish 5.24 receptor (Kuang *et al.*, 2003). From this homology modelling and from alignments with other family C GPCRs, the residues known to interact with the α-amino acid moiety of L-Glu in $mGlu_1$ are clearly conserved in GPRC6A (Table 1). The modelled L-α-amino acid recognition site was validated as mutations S149A and T172A (corresponding to S165 and T188 in $mGlu_1$) completely obliterated receptor activity in response to amino acids (Wellendorph *et al.*, 2005). Importance of these residues is substantiated by mutagenesis studies conducted on the homologous goldfish receptor 5.24, in that mutation of all the five highly conserved residues known to bind to the α-amino acid moiety (Table 1) also dramatically disturbs activity of this receptor (Kuang *et al.*, 2003; Luu *et al.*, 2004). Furthermore, in the 5.24 receptor, two acidic residues (D388 and E47) have been confirmed to be directly involved in binding of the distal basic guanidinium group of L-Arg. The precise environment of the distal end of the GPRC6A-binding pocket is less clear and the residue(s) responsible for binding the positively charged distal end of the amino acid have not been identified. The equivalent of D388 is not conserved to GPRC6A and in general the sequences are quite divergent in this region. Possibly, this could reflect the fact that GPRC6A is less selective for basic amino acids than 5.24. Based on our homology model, residues E67, E170, D401 and D403 are potential candidates for interacting with the distal end of positively charged ligands (Wellendorph *et al.*, 2005) but remain to be investigated by site-directed mutagenesis. Finally, alternative scenarios for the amino acid-binding pocket in GPRC6A might be relevant to consider, such as the use of the lysine-arginine-ornithine-binding protein (Kang *et al.*, 1991; Oh *et al.*, 1993) as template, in which L-Lys binds in a more folded conformation than L-Glu in $mGlu_1$ (Wellendorph *et al.*, 2005).

Molecular pharmacology of the T1R1+T1R3 taste receptor

The T1R class of GPCRs consists of three subunits: T1R1, T1R2 and T1R3 that all carry the typical family C characteristics of a large VFT domain, a CRD and a 7TM domain. T1R subunits are selectively expressed in taste buds (Hoon *et al.*, 1999) where they combine to form heterodimeric taste receptors, responding to either sweet stimuli (T1R2+T1R3) or amino

acids/umami taste (T1R1+T1R3) (Nelson *et al.*, 2001; 2002; Li *et al.*, 2002). In order to form functional receptors, the individual subunits T1R1 and T1R2 depend on co-expression and dimerization with T1R3, thus comparable to the $GABA_B$ receptor in being obligatory heterodimers. This functional prerequisite is underlined by studies using chimeric receptors and knockout animals (Zhao *et al.*, 2003; Xu *et al.*, 2004). Given the topic of this review, the T1R1+T1R3 amino acid receptor is highlighted. Being an L-amino acid-sensing receptor, the conservation of the five key residues involved in binding of the α-amino acid moiety of L-Glu in $mGlu_1$ is not unexpected. The signalling of T1R1+T1R3 is augmented dramatically by 5′-inosine monophospate (IMP), which is a hallmark of umami taste (Yamaguchi, 1991). Indeed, in the presence of IMP, the receptor responds to all natural L-amino acids but L-Trp (Figure 4), as well as to the synthetic L-Glu analogue L-AP4 (Li *et al.*, 2002; Nelson *et al.*, 2002). Because L-Glu and IMP have no effect on the T1R2+T1R3 sweet taste receptor, it is inferred that these bind to the T1R1 subunit (Li *et al.*, 2002; Xu *et al.*, 2004).

Whereas the $mGlu_1$ crystal has formed the basis for homology models of T1R2 and T1R3 (reviewed by Cui *et al.*, 2006), no model exists for T1R1, which is the subunit believed to harbour the predicted amino acid-binding pocket. Consequently and additionally, no mutations in the presumed binding pocket have been reported and details about the molecular recognition of amino acids in the umami receptor remain to be addressed. From work by Silve *et al.*, it seems plausible that T1R1 and/or T1R3 also contain a Ca^{2+}-binding site as residues known to involve Ca^{2+} binding in CaR are conserved to these receptor subunits (Silve *et al.*, 2005). So far, no molecular evidence has emerged to support this view, but recently the involvement of T1R3 in calcium-magnesium taste has been implied based on taste studies *in vivo* (Tordoff *et al.*, 2008), also proposing that T1R3 could have a dimerization partner with Ca^{2+}-sensing properties such as CaR. As delineated above, another potential partner for dimerization is GPRC6A, which intriguingly is also expressed in taste buds (Wellendorph *et al.*, 2007).

Conclusion and outlook

As we have outlined in the present review, the last decade has increased our knowledge of how amino acids bind to the VFTs of family C receptors dramatically. These insights have provided a basis to understand subtype selectivity of mGlu receptors and the initial events in receptor activation. Interestingly, whereas mGlu and $GABA_B$ receptors are exclusively activated by one endogenous agonist, CaR, T1R1 and GPRC6A are promiscuously activated/modulated by a range of L-α-amino acids (Figure 4). The molecular mechanism of this promiscuity and the physiological importance of the individual amino acids still remains to be shown. Also the molecular mechanism of cation activation/modulation of family C remains to be fully uncovered. Structural and biophysical studies have unravelled the initial events in activation of the dimeric receptor by closing of the VFT around the agonist, but how this is translated into activation of the 7TM domains is still a mystery. Is it contraction of the two 7TM domains in the dimer, a conformational change in the individual 7TM domains induced via the rigid CRD, or a third mechanism? These and other important unsolved questions ensure that the next decade of family C receptor research will be exciting to follow.

Acknowledgements

We would like to thank all present and past members of our group working on family C receptors for their contributions. Currently, our work on family C receptors is supported by the Danish Medical Research Council, the Drug Research Academy, the Villum Kann Rasmussen Foundation, Simon Foughner Hartmanns Familiefond, the Aase and Ejner Danielsen Foundation and the Alfred Benzon Foundation.

Conflict of interest

None.

References

Abe H, Tateyama M, Kubo Y (2003). Functional identification of Gd^{3+} binding site of metabotropic glutamate receptor 1a. *FEBS Lett* **545**: 233–238.

Acher FC, Bertrand H-O (2005). Amino acid recognition by Venus flytrap domains is encoded in an 8-residue motif. *Biopolymers* **80**: 357–366.

Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Bai M, Quinn S, Trivedi S, Kifor O, Pearce SHS, Pollak MR *et al.* (1996). Expression and characterization of inactivating and activating mutations in the human Ca^{2+}o-sensing receptor. *J Biol Chem* **271**: 19537–19545.

Bai M, Trivedi S, Kifor O, Quinn SJ, Brown EM (1999). Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. *Proc Natl Acad Sci USA* **96**: 2834–2839.

Bessis AS, Rondard P, Gaven F, Brabet I, Triballeau N, Prézeau L *et al.* (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 USA* **99**: 11097–11102.

Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004). Molecular structure and physiological functions of $GABA_B$ receptors. *Physiol Rev* **84**: 835–867.

Bhave G, Nadin BM, Brasier DJ, Glauner KS, Shah RD, Heinemann SF *et al.* (2003). Membrane topology of a metabotropic glutamate receptor. *J Biol Chem* **278**: 30294–30301.

Bjarnadóttir TK, Fredriksson R, Schiöth HB (2005). The gene repertoire and the common evolutionary history of glutamate, pheromone (V2R), taste(1) and other related G protein-coupled receptors. *Gene* **362**: 70–84.

Bjarnadóttir TK, Gloriam DE, Hellstrand SH, Kristiansson H, Fredriksson R, Schiöth HB (2006). Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. *Genomics* **88**: 263–273.

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J *et al.* (1980). Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283**: 92–94.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M *et al*. (2002). International Union of Pharmacology. XXXIII. Mammalian γ-aminobutyric acid$_B$ receptors: structure and function. *Pharmacol Rev* **54**: 247–264.

Bräuner-Osborne H, Krogsgaard-Larsen P (1999). Functional pharmacology of cloned heterodimeric GABA$_B$ receptors expressed in mammalian cells. *Br J Pharmacol* **128**: 1370–1374.

Bräuner-Osborne H, Krogsgaard-Larsen P (2000). Sequence and expression pattern of a novel human orphan G-protein coupled receptor GPRC5B, a family C receptor with a short amino terminal domain. *Genomics* **65**: 121–128.

Bräuner-Osborne H, Jensen AA, Sheppard PO, O'Hara P, Krogsgaard-Larsen P (1999). The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain. *J Biol Chem* **274**: 18382–18386.

Bräuner-Osborne H, Egebjerg J, Nielsen E, Madsen U, Krogsgaard-Larsen P (2000). Ligands for glutmate receptors: Design and therapeutic prospects. *J Med Chem* **43**: 2609–2645.

Bräuner-Osborne H, Jensen AA, Sheppard PO, Brodin B, Krogsgaard-Larsen P, O'Hara P (2001). Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D. *Biochim Biophys Acta* **1518**: 237–248.

Bräuner-Osborne H, Wellendorph P, Jensen AA (2007). Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Curr Drug Targets* **8**: 169–184.

Brock C, Oueslati N, Soler S, Boudier L, Rondard P, Pin J-P (2007). Activation of a dimeric metabotropic glutamate receptor by intersubunit rearrangement. *J Biol Chem* **282**: 33000–33008.

Brown EM (2007). Clinical lessons from the calcium-sensing receptor. *Nat Clin Pract Endocrinol Metab* **3**: 122–133.

Brown EM, MacLeod RJ (2001). Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81**: 239–297.

Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O *et al*. (1993). Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. *Nature* **366**: 575–580.

Busque SM, Kerstetter JE, Geibel JP, Insogna K (2005). L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. *Am J Physiol* **289**: G664–G669.

Calver AR, Michalovich D, Testa TT, Robbins MJ, Jaillard C, Hill J *et al*. (2003). Molecular cloning and characterisation of a novel GABA$_B$-related G-protein coupled receptor. *Mol Brain Res* **110**: 305–317.

Cheng Y, Lotan R (1998). Molecular cloning and characterization of a novel retinoic acid-inducible gene that encodes a putative G protein-coupled receptor. *J Biol Chem* **273**: 35008–35015.

Christiansen B, Wellendorph P, Bräuner-Osborne H (2006a). Activity of L-α-amino acids at the promiscuous goldfish odorant receptor 5.24. *Eur J Pharmacol* **536**: 98–101.

Christiansen B, Wellendorph P, Bräuner-Osborne H (2006b). Known regulators of nitric oxide synthase and arginase are agonists at the human G-protein-coupled receptor GPRC6A. *Br J Pharmacol* **147**: 855–863.

Christiansen B, Hansen KB, Wellendorph P, Bräuner-Osborne H (2007). Pharmacological characterization of mouse GPRC6A, an L-α-amino acid receptor with ability to sense divalent cations. *Br J Pharmacol* **150**: 798–807.

Conigrave AD, Brown EM (2006). Taste receptors in the gastrointestinal tract. II. L-amino acid sensing by calcium-sensing receptors: implications for GI physiology. *Am J Physiol* **291**: G753–761.

Conigrave AD, Quinn SJ, Brown EM (2000). L-amino acid sensing by the extracellular Ca^{2+}-sensing receptor. *Proc Natl Acad Sci USA* **97**: 4814–4819.

Conigrave AD, Mun HC, Delbridge L, Quinn SJ, Wilkinson M, Brown EM (2004). L-amino acids regulate parathyroid hormone secretion. *J Biol Chem* **279**: 38151–38159.

Conigrave AD, Mun HC, Brennan SC (2007). Physiological significance of L-amino acid sensing by extracellular Ca^{2+}-sensing receptors. *Biochem Soc Trans* **35**: 1195–1198.

Conigrave AD, Brown EM, Rizzoli R (2008). Dietary protein and bone health: Roles of amino acid-sensing receptors in the control of calcium metabolism and bone homeostasis. *Annu Rev Nutr* **28**: 131–155.

Costantino G, Macchiarulo A, Guadix AE, Pellicciari R (2001). QSAR and molecular modeling studies of baclofen analogues as GABA$_B$ agonists. Insights into the role of the aromatic moiety in GABA$_B$ binding and activation. *J Med Chem* **44**: 1827–1832.

Coulombe J, Faure H, Robin B, Ruat M (2004). In vitro effects of strontium ranelate on the extracellular calcium-sensing receptor. *Biochem Biophys Res Commun* **323**: 1184–1190.

Cui M, Jiang P, Maillet E, Max M, Margolskee RF, Osman R (2006). The heterodimeric sweet taste receptor has multiple potential ligand binding sites. *Curr Pharm Des* **12**: 4591–4600.

Fan GF, Ray K, Zhao XM, Goldsmith PK, Spiegel AM (1998). Mutational analysis of the cysteines in the extracellular domain of the human Ca^{2+} receptor: effects on cell surface expression, dimerization and signal transduction. *FEBS Lett* **436**: 353–356.

Frauli M, Hubert N, Schann S, Triballeau N, Bertrand H-O, Acher F, Neuville P, Pin J-P, Prézeau L (2007). Amino-pyrrolidine tricarboxylic acids give new insight into group III metabotropic glutamate receptor activation mechanism. *Molecular Pharmacology* **71**: 704–712.

Frauli M, Neuville P, Vol C, Pin J-P, Prezéau L (2006). Among the twenty classical L-amino acids, only glutamate directly activates metabotropic glutamate receptors. *Neuropharmacology* **50**: 245–253.

Galvez T, Parmentier ML, Joly C, Malitschek B, Kaupmann K, Kuhn R *et al*. (1999). Mutagenesis and modeling of the GABA$_B$ receptor extracellular domain support a Venus flytrap mechanism for ligand binding. *J Biol Chem* **274**: 13362–13369.

Galvez T, Prézeau L, Milioti G, Franek M, Joly C, Froestl W *et al*. (2000a). Mapping the agonist-binding site of GABA$_B$ type 1 subunit sheds light on the activation process of GABA$_B$ receptors. *J Biol Chem* **275**: 41166–41174.

Galvez T, Urwyler S, Prézeau L, Mosbacher J, Joly C, Malitschek B *et al*. (2000b). Ca^{2+} requirement for high-affinity γ-aminobutyric acid (GABA) binding at GABA$_B$ receptors: involvement of serine 269 of the GABA$_B$R1 subunit. *Mol Pharmacol* **57**: 419–426.

Gasparini F, Spooren W (2007). Allosteric modulators for mGlu receptors. *Curr Neuropharmacology* **5**: 187–194.

Gorodkin J, Heyer LJ, Brunak S, Stormo GD (1997). Displaying the information contents of structural RNA alignments: the structure logos. *Comput Appl Biosci* **13**: 583–586.

Hammerland LG, Krapcho KJ, Garrett JE, Alasti N, Hung BC, Simin RT *et al*. (1999). Domains determining ligand specificity for Ca^{2+} receptors. *Mol Pharmacol* **55**: 642–648.

Hampson DR, Huang X-P, Pekhletski R, Peltekova V, Hornby G, Thomsen C, Thøgersen H (1999). Probing the ligand-binding domain of the mGluR4 subtype of metabotropic glutamate receptor. *J Biol Chem* **274**: 33488–33495.

Hauache OM, Hu J, Ray K, Spiegel AM (2000). Functional interactions between the extracellular domain and the seven-transmembrane domain in Ca^{2+} receptor activation. *Endocrine* **13**: 63–70.

Hermit MB, Greenwood JR, Nielsen B, Bunch L, Jørgensen CG, Vestergaard HT *et al*. (2004). Ibotenic acid and thioibotenic acid: a remarkable difference in activity at group III metabotropic glutamate receptors. *Eur J Pharmacol* **486**: 241–250.

Hill DR, Bowery NG (1981). ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive GABA$_B$ sites in rat brain. *Nature* **290**: 149–152.

Hira T, Nakajima S, Eto Y, Hara H (2008). Calcium-sensing receptor mediates phenylalanine-induced cholecystokinin secretion in enteroendocrine STC-1 cells. *FEBS J*.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS (1999).

Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* **96**: 541–551.

Hrabák A (2006). Common ligands of G-protein-coupled receptors and arginine-utilizing enzymes. *Br J Pharmacol* **147**: 835–837.

Hu J, Spiegel AM (2007). Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators. *J Cell Mol Med* **11**: 908–922.

Hu J, Reyes-Cruz G, Chen W, Jacobson KA, Spiegel AM (2002). Identification of acidic residues in the extracellular loops of the seven-transmembrane domain of the human Ca^{2+} receptor critical for response to Ca^{2+} and a positive allosteric modulator. *J Biol Chem* **277**: 46622–46631.

Hu J, McLarnon SJ, Mora S, Jiang J, Thomas C, Jacobson KA *et al.* (2005). A region in the seven-transmembrane domain of the human Ca^{2+} receptor critical for response to Ca^{2+}. *J Biol Chem* **280**: 5113–5120.

Huang Y, Zhou Y, Yang W, Butters R, Lee HW, Li S *et al.* (2007). Identification and dissection of Ca^{2+}-binding sites in the extracellular domain of Ca^{2+}-sensing receptor. *J Biol Chem* **282**: 19000–19010.

Jensen AA, Bräuner-Osborne H (2007). Allosteric modulation of the calcium-sensing receptor. *Curr Neuropharmacology* **5**: 180–186.

Jensen AA, Spalding TA, Burstein ES, Sheppard PO, O'Hara PJ, Brann MR *et al.* (2000). Functional importance of the Ala^{116}-Pro^{136} region in the calcium-sensing receptor. Constitutive activity and inverse agonism in a family C G-protein coupled receptor. *J Biol Chem* **275**: 29547–29555.

Jensen AA, Madsen BE, Krogsgaard-Larsen P, Bräuner-Osborne H (2001a). Pharmacological characterization of homobaclofen on wild type and mutant $GABAB_{1b}$ receptors coexpressed with the $GABA_{B2}$ receptor. *Eur J Pharmacol* **417**: 177–180.

Jensen AA, Sheppard PO, Jensen LB, O'Hara PJ, Bräuner-Osborne H (2001b). Construction of a high affinity zinc binding site in the metabotropic glutamate receptor mGluR1. Non-competitive antagonism originating from the amino terminal domain of a family C G-protein-coupled receptor. *J Biol Chem* **276**: 10110–10118.

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M *et al.* (1998). $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. *Nature* **396**: 674–679.

Kammermeier PJ, Yun J (2005). Activation of metabotropic glutamate receptor 1 dimers requires glutamate binding in both subunits. *J Pharmacol Exp Ther* **312**: 502–508.

Kang CH, Shin WC, Yamagata Y, Gokcen S, Ames GF, Kim SH (1991). Crystal structure of the lysine-, arginine-, ornithine-binding protein (LAO) from Salmonella typhimurium at 2.7-Å resolution. *J Biol Chem* **266**: 23893–23899.

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ *et al.* (1997). Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386**: 239–246.

Kaupmann K, Malitschek B, Schuler V, Heid J, Fröstl W, Beck P *et al.* (1998). $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396**: 683–687.

Kniazeff J, Galvez T, Labesse G, 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**: 7352–7361.

Kniazeff J, Bessis AS, Maurel D, Ansanay H, Prézeau L, Pin JP (2004a). Closed state of both binding domains of homodimeric mGlu receptors is required for full activity. *Nat Struct Mol Biol* **11**: 706–713.

Kniazeff J, Saintot PP, Goudet C, Liu J, Charnet A, Guillon G *et al.* (2004b). Locking the dimeric $GABA_B$ G-protein-coupled receptor in its active state. *J Neurosci* **24**: 370–377.

Kolakowski LF Jr. (1994). GCRDb: a G-protein-coupled receptor database. *Receptors Channels* **2**: 1–7.

Kuang D, Yao Y, Lam J, Tsushima RG, Hampson DR (2005). Cloning and characterization of a family C orphan G-protein coupled receptor. *J Neurochem* **93**: 383–391.

Kuang D, Yao Y, Wang M, Pattabiraman N, Kotra LP, Hampson DR (2003). Molecular similarities in the ligand binding pockets of an odorant receptor and the metabotropic glutamate receptors. *J Biol Chem* **278**: 42551–42559.

Kubo Y, Miyashita T, Murata Y (1998). Structural Basis for a Ca^{2+}-Sensing Function of the Metabotropic Glutamate Receptors. *Science* **279**: 1722–1725.

Kuner R, Köhr G, Grünewald S, Eisenhardt G, Bach A, Kornau H-C (1999). Role of heteromer formation in $GABA_B$ receptor function. *Science* **283**: 74–77.

Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T *et al.* (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **407**: 971–977.

Lee HJ, Mun HC, Lewis NC, Crouch MF, Culverston EL, Mason RS *et al.* (2007). Allosteric activation of the extracellular Ca^{2+}-sensing receptor by L-amino acids enhances ERK1/2 phosphorylation. *Biochem J* **404**: 141–149.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E (2002). Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* **99**: 4692–4696.

Lundstrom K (2005). The future of G protein-coupled receptors as targets in drug discovery. *IDrugs* **8**: 909–913.

Luu P, Acher F, Bertrand HO, Fan J, Ngai J (2004). Molecular determinants of ligand selectivity in a vertebrate odorant receptor. *J Neurosci* **24**: 10128–10137.

Madsen U, Bräuner-Osborne H, Greenwood JR, Johansen TN, Krogsgaard-Larsen P, Liljefors T *et al.* (2005). GABA and glutamate receptor ligands and their therapeutic potential in CNS disorders. In: Gad SC (ed.). *Drug Discovery Handbook*. Wiley: New York, pp. 797–907.

Malherbe P, Knoflach F, Broger C, Ohresser S, Kratzeisen C, Adam G, Stadler H, Kemp JA, Mutel V (2001). Identification of essential residues involved in the glutamate binding pocket of the group II metabotropic glutamate receptor. *Mol Pharmacol* **60**: 944–954.

Malitschek B, Ruegg D, Heid J, Kaupmann K, Bittiger H, Fröstl W *et al.* (1998). Developmental changes of agonist affinity at $GABA_BR1$ receptor variants in rat brain. *Mol Cell Neurosci* **12**: 56–64.

Mun HC, Culverston EL, Franks AH, Collyer CA, Clifton-Bligh R, Conigrave AD (2005). A double mutation in the extracellular Ca^{2+}-sensing receptor's Venus fly trap domain that selectively disables L-amino acid sensing. *J Biol Chem* **280**: 29067–29072.

Mun HC, Franks AH, Culverston EL, Krapcho K, Nemeth EF, Conigrave AD (2004). The Venus Fly Trap domain of the extracellular Ca^{2+}-sensing receptor is required for L-amino acid sensing. *J Biol Chem* **279**: 51739–51744.

Muto T, Tsuchiya D, Morikawa K, Jingami H (2007). Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proc Natl Acad Sci USA* **104**: 3759–3764.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS (2001). Mammalian sweet taste receptors. *Cell* **106**: 381–390.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJP *et al.* (2002). An amino-acid taste receptor. *Nature* **416**: 199–202.

Nemeth EF, Steffey ME, Hammerland LG, Hung BCP, Van Wagenen BC, DelMar EG *et al.* (1998). Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *Proc Natl Acad Sci USA* **95**: 4040–4045.

Nemeth EF, DelMar EG, Heaton WL, Miller MA, Lambert LD, Conklin RL *et al.* (2001). Calcilytic compounds: potent and selective Ca^{2+} receptor antagonists that stimulate secretion of parathyroid hormone. *J Pharmacol Exp Ther* **299**: 323–331.

Nomura R, Suzuki Y, Kakizuka A, Jingami H (2008). Direct detection of the interaction between recombinant soluble extracellular regions in the heterodimeric metabotropic γ-aminobutyric acid receptor. *J Biol Chem* **283**: 4665–4673.

O'Hara PJ, Sheppard PO, Thøgersen H, Venezia D, Haldeman BA,

McGrane V, Houamed KM, Thomsen C, Gilbert TL, Mulvihill ER (1993). The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* **11**: 41–52.

Oh BH, Pandit J, Kang CH, Nikaido K, Gokcen S, Ames GF *et al*. (1993). Three-dimensional structures of the periplasmic lysine/arginine/ornithine-binding protein with and without a ligand. *J Biol Chem* **268**: 11348–11355.

Pace AJ, Gama L, Breitwieser GE (1999). Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys -> Ser mutations at Cys101 and Cys236. *J Biol Chem* **274**: 11629–11634.

Pearce SH, Bai M, Quinn SJ, Kifor O, Brown EM, Thakker RV (1996). Functional characterization of calcium-sensing receptor mutations expressed in human embryonic kidney cells. *J Clin Invest* **98**: 1860–1866.

Pérez-Garci E, Gassmann M, Bettler B, Larkum ME (2006). The $GABA_{B1b}$ isoform mediates long-lasting inhibition of dendritic Ca^{2+} spikes in layer 5 somatosensory pyramidal neurons. *Neuron* **50**: 603–616.

Pi M, Faber P, Ekema G, Jackson PD, Ting A, Wang N *et al*. (2005). Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem* **280**: 40201–40209.

Pidasheva S, Grant M, Canaff L, Ercan O, Kumar U, Hendy GN (2006). Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of CASR mutants retained intracellularly. *Hum Mol Genet* **15**: 2200–2209.

Pierce KL, Premont RT, Lefkowitz RJ (2002). Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* **3**: 639–650.

Pin J-P, Prézeau L (2007). Allosteric modulators of $GABA_B$ receptors: Mechanism of action and therapeutic perspective. *Curr Neuropharmacol* **5**: 195–201.

Pin JP, Kniazeff J, Binet V, Liu J, Maurel D, Galvez T *et al*. (2004). Activation mechanism of the heterodimeric $GABA_B$ receptor. *Biochem Pharmacol* **68**: 1565–1572.

Quinn SJ, Bai M, Brown EM (2004). pH Sensing by the calcium-sensing receptor. *J Biol Chem* **279**: 37241–37249.

Quinn SJ, Kifor O, Trivedi S, Diaz R, Vassilev P, Brown E (1998). Sodium and ionic strength sensing by the calcium receptor. *J Biol Chem* **273**: 19579–19586.

Quinn SJ, Ye CP, Diaz R, Kifor O, Bai M, Vassilev P *et al*. (1997). The Ca^{2+}-sensing receptor: A target for polyamines. *Am J Physiol* **273**: C1315–C1323.

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC *et al*. (2007). Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature* **450**: 383–387.

Ray K, Northup J (2002). Evidence for distinct cation and calcimimetic compound (NPS 568) recognition domains in the transmembrane regions of the human Ca^{2+} receptor. *J Biol Chem* **277**: 18908–18913.

Ritzén A, Mathiesen JM, Thomsen C (2005). Molecular pharmacology and therapeutic prospects of metabotropic glutamate receptor allosteric modulators. *Basic Clin Pharmacol Toxicol* **9**: 202–213.

Robbins MJ, Milchalovich D, Hill J, Calver AR, Medhurst AD, Gloger I *et al*. (2000). Molecular cloning and characterization of two novel retinoic acid-inducible orphan G-protein-coupled receptors (GPRC5B and GPRC5C). *Genomics* **67**: 8–18.

Romano C, Miller JK, Hyrc K, Dikranian S, Mennerick S, Takeuchi Y *et al*. (2001). Covalent and noncovalent interactions mediate metabotropic glutamate receptor $mGlu_5$ dimerization. *Mol Pharmacol* **59**: 46–53.

Rondard P, Huang S, Monnier C, Tu H, Blanchard B, Oueslati N *et al*. (2008). Functioning of the dimeric $GABA_B$ receptor extracellular domain revealed by glycan wedge scanning. *EMBO J* **27**: 1321–1332.

Rondard P, Liu J, Huang S, Malhaire F, Vol C, Pinault A *et al*. (2006). Coupling of agonist binding to effector domain activation in metabotropic glutamate-like receptors. *J Biol Chem* **281**: 24653–24661.

Rosemond E, Peltekova V, Naples M, Thøgersen H, Hampson DR (2002). Molecular determinants of high affinity binding to group III metabotropic glutamate receptors. *J Biol Chem* **277**: 7333–7340.

Rosemond E, Wang M, Yao Y, Storjohann L, Stormann T, Johnson EC *et al*. (2004). Molecular basis for the differential agonist affinities of group III metabotropic glutamate receptors. *Mol Pharmacol* **66**: 834–842.

Ruat M, Snowman AM, Hester LD, Snyder SH (1996). Cloned and expressed rat Ca^{2+}-sensing receptor. *J Biol Chem* **271**: 5972–5975.

Sato T, Shimada Y, Nagasawa N, Nakanishi S, Jingami H (2003). Amino acid mutagenesis of the ligand binding site and the dimmer interface of the metabotropic glutamate receptor 1. Identificaiton of crucial residuces for setting the activated state. *J Biol Chem* **278**: 4314–4321.

Schneider TD, Stephens RM (1990). Sequence logos: a new way to display consensus sequences. *Nucl Acids Res* **18**: 6097–6100.

Schoepp DD, Jane DE, Monn JA (1999). Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **38**: 1431–1476.

Silve C, Petrel C, Leroy C, Bruel H, Mallet E, Rognan D *et al*. (2005). Delineating a Ca^{2+} binding pocket within the venus flytrap module of the human calcium-sensing receptor. *J Biol Chem* **280**: 37917–37923.

Speca DJ, Lin DM, Sorensen PW, Isacoff EY, Ngai J, Dittman AH (1999). Functional identification of a goldfish odorant receptor. *Neuron* **23**: 487–498.

Tateyama M, Abe H, Nakata H, Saito O, Kubo Y (2004). Ligand-induced rearrangement of the dimeric metabotropic glutamate receptor 1α *Nat Struct Mol Biol* **11**: 637–642.

Tfelt-Hansen J, Brown EM (2005). The calcium-sensing receptor in normal physiology and pathophysiology: a review. *Crit Rev Clin Lab Sci* **42**: 35–70.

Tordoff MG, Shao H, Alarcon LK, Margolskee RF, Mosinger B, Bachmanov AA *et al*. (2008). Involvement of T1R3 in calcium-magnesium taste. *Physiol Genomics* **34**: 338–348.

Trivedi R, Mithal A, Chattopadhyay N (2008). Recent updates on the calcium-sensing receptor as a drug target. *Curr Med Chem* **15**: 178–186.

Tsuchiya D, Kunishima N, Kamiya N, Jingami H, 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 USA* **99**: 2660–2665.

Tsuji Y, Shimada Y, Takeshita T, Kajimura N, Nomura S, Sekiyama N *et al*. (2000). Cryptic dimer interface and domain organization of the extracellular region of metabotropic glutamate receptor subtype 1. *J Biol Chem* **275**: 28144–28151.

Vigot R, Barbieri S, Bräuner-Osborne H, Turecek R, Shigemoto R, Zhang YP *et al*. (2006). Differential compartmentalization and distinct functions of $GABA_B$ receptor variants. *Neuron* **50**: 589–601.

Wang M, Yao Y, Kuang D, Hampson DR (2006). Activation of family C G-protein-coupled receptors by the tripeptide glutathione. *J Biol Chem* **281**: 8864–8870.

Wellendorph P, Bräuner-Osborne H (2004). Molecular cloning, expression, and sequence analysis of GPRC6A, a novel family C G-protein-coupled receptor. *Gene* **335**: 37–46.

Wellendorph P, Hansen KB, Balsgaard A, Greenwood JR, Egebjerg J, Bräuner-Osborne H (2005). Deorphanization of GPRC6A: a promiscuous L-α-amino acid receptor with preference for basic amino acids. *Mol Pharmacol* **67**: 589–597.

Wellendorph P, Burhenne N, Christiansen B, Walter B, Schmale H, Bräuner-Osborne H (2007). The rat GPRC6A: cloning and characterization. *Gene* **396**: 257–267.

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH *et al*. (1998). Heterodimerization is required for the formation of a functional $GABA_B$ receptor. *Nature* **396**: 679–682.

Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X (2004). Different

functional roles of T1R subunits in the heteromeric taste receptors. *Proc Natl Acad Sci USA* **101**: 14258–14263.

Yamaguchi S (1991). Basic properties of umami and effects on humans. *Physiol Behav* **49**: 833–841.

Yao Y, Pattabiraman N, Michne WF, Huang XP, Hampson DR (2003). Molecular modeling and mutagenesis of the ligand-binding pocket of the mGlu3 subtype of metabotropic glutamate receptor. *J Neurochem* **86**: 947–957.

Ye C, Ho-Pao CL, Kanazirska M, Quinn S, Rogers K, Seidman CE *et al*. (1997). Amyloid-beta proteins activate Ca^{2+}-permeable channels through calcium-sensing receptors. *J Neurosci Res* **47**: 547–554.

Zhang Z, Qiu W, Quinn SJ, Conigrave AD, Brown EM, Bai M (2002). Three adjacent serines in the extracellular domains of the CaR are required for L-amino acid-mediated potentiation of receptor function. *J Biol Chem* **277**: 33727–33735.

Zhang Z, Sun S, Quinn SJ, Brown EM, Bai M (2001). The extracellular calcium-sensing receptor dimerizes through multiple types of intermolecular interactions. *J Biol Chem* **276**: 5316–5322.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ *et al*. (2003). The receptors for mammalian sweet and umami taste. *Cell* **115**: 255–266.