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European Journal of Pharmacology 375 (1999) 277–294

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Review

New perspectives for the development of selective metabotropic glutamate receptor ligands

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Accepted 30 April 1999

Abstract

The metabotropic glutamate receptors are GTP-binding-protein (G-protein) coupled receptors that play important roles in regulating the activity of many synapses in the central nervous system. As such, these receptors are involved in a wide number of physiological and pathological processes. Within the last few years, new potent and selective agonists and antagonists as well as radioligands acting on these receptors have been developed. Molecular modeling studies revealed the structural features of the glutamate binding site, and will be useful for the design of more selective and potent ligands. More interestingly, recent data revealed new regulatory sites on the receptor protein, able either to decrease or potentiate the action of the endogenous ligand. No doubt that in the near future a multitude of new tools to modulate the activity of these receptors will be discovered, enabling the identification of the possible therapeutic applications for these new neuroactive molecules.

Keywords: Metabotropic glutamate receptor ligand; G-protein coupling domain; Receptor activation; Regulating site; Molecular modeling; Neuroactive molecule

1. Introduction

Although glutamate is the neurotransmitter of a vast majority of synapses in the central nervous system, there are actually no drug acting on glutamate receptors with a clear therapeutic application. For many years, glutamate was assumed to act exclusively on ionotropic glutamate receptors (the AMPA, *N*-methyl-D-aspartate and kainate receptors). Among these, the AMPA and *N*-methyl-D-aspartate receptors which have been the subject of intense research by drug developers, play such a crucial role in the fast excitatory transmission that a tune modulation of their activity is very difficult to achieve without side effects. The kainate receptors, some new roles of which have been recently unraveled, constitute a possible new target for drugs modulating the activity of the glutamatergic system (Lerma et al., 1997; Mulle et al., 1998). In addition, the discovery of a family of GTP-binding-protein (G-protein) coupled glutamate receptors opened a new area of research with new possibilities of modulating fast excitatory synaptic transmission (Conn and Pin, 1997).

These metabotropic glutamate (mGlu) receptors were first characterized as phospholipase C-coupled receptors, and then additional subtypes negatively coupled to adenylyl cyclase were identified both pharmacologically and via the cloning of their cDNA. Eight mGlu receptors subtypes have been identified that can be subdivided into three groups based on their sequence similarity (Conn and Pin, 1997). Group-I is composed of $mGlu_1$ and $mGlu_5$ receptors which activate phospholipase C, whereas group-II ($mGlu_2$ and $mGlu_3$ receptors) and group-III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors) can inhibit adenylyl cyclase activity (Table 1). Many of these receptors exist as various isoforms with different intracellular carboxy-termini generated by alternative splicing of their pre-messenger RNA (Conn and Pin, 1997; Corti et al., 1998) (Table 1).

These receptors have been localized either pre- or post-synaptically at most (if not all) glutamatergic synapses, and at some γ-aminobutyric acid (GABA)-ergic synapses. In most instances, group-I mGlu receptors increase cell

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00258-7

Table 1
Classification, transduction and pharmacology of the mGluR subtypes

Group	Subtypes	Splice Variants	Transduction	General Agonist	Group Selective Agonists	Subtype Selective Agonists	General Antagonist	Group Selective Antagonists	Subtype Selective Antagonists
I	mGluR1	a, b, c, d	+ PLC + Ca-K; - K - VSCC + L-VSCC	ABHxD-I	Quisqualate DHPG	CHPG	ACPT-II	LY393675 NPS 2390	CBPG, CPCCOEt LY367385 MPEP
	mGluR5	a, b							
II	mGluR2		- AC - VSCC		1S,3S-ACPD 2R,4R-APDC LY354740 LY379268	NAAG		MCCG-I, PCCG4 LY307452 LY341495 (EGlu)	
	mGluR3								
III	mGluR4	a, b	- AC - VSCC + K		L-AP4 L-SOP CPrAP4 ACPT-I (+) ACPT-III PPG	S-homoAMPA		MAP4	
	mGluR6								
	mGluR7	a, b							
	mGluR8	a, b							

The various mGlu receptor subtypes, their classification in three groups, their transduction mechanisms and their selective agonists and antagonists. For the abbreviations not defined in the text, see the legend to Table 2.

excitability by inhibiting the activity of K^+ channels (Conn and Pin, 1997), but some inhibitory actions resulting from the activation of Ca^{2+}-activated K^+ channels have been observed (Fagni et al., 1991; Fiorillo and Williams, 1998). Although group-I mGlu receptors are mostly located in post-synaptic elements, pre-synaptic actions of these receptors have been reported (Gereau and Conn, 1995; Manzoni et al., 1995). In most cases, they have been shown to decrease neurotransmitter release probably by inhibiting Ca^{2+} channels (Conn and Pin, 1997), but a potentiation of glutamate release has also been observed (Herrero et al., 1998). Group-I mGlu receptors have been shown to facilitate glutamate-induced neuronal toxicity, and to participate in pain sensitivity (Conn and Pin, 1997). Accordingly, potential therapeutic applications of group-I antagonists are expected. Group-II and group-III mGlu receptors are mostly located on glutamatergic terminals and inhibit the release process (Conn and Pin, 1997). Accordingly, agonists for these receptor types are expected to have many potential therapeutic applications by inhibiting the glutamatergic system. These include, for example, protection from excitotoxicity (Bond et al., 1998), treatment of anxiety (Helton et al., 1998), Parkinson's disease (Konieczny et al., 1998), schizophrenia (Moghaddam and Adams, 1998) and drug addiction (Helton et al., 1997).

The aim of the present review is to outline the recent development of the mGlu receptor pharmacology. We will first summarize our knowledge on the structure-function relationship of these receptor molecules since this is essential to understand the mechanisms of action of agonists, antagonists and allosteric regulators. Then, we will describe the recently identified selective agonists and antagonists. Finally, we will discuss how all these new information can be used for the development of new compounds acting on mGlu receptors.

2. The original structure of mGlu receptors

The cloning of the mGlu receptor cDNAs revealed they share sequence similarity neither with the large family of the rhodopsin-like G-protein coupled receptors (now referred as the family 1) nor with the large-peptide receptors (family 2, receptors for the vasoactive intestinal polypeptide glucagon, . . .) (Conn and Pin, 1997; Bockaert and Pin, 1999). These receptors constitute therefore a new family of G-protein coupled receptors (family 3) that also includes the Ca^{2+}-sensing receptor, some vomeronasal receptors and the GABA type-B receptor ($GABA_B$ receptors) (Bockaert and Pin, 1999). The present chapter outlines the specific structural features of these family 3 receptors.

2.1. The binding domain

Family 3 receptors possess a surprisingly large N-terminal extracellular domain of 500 to 600 residues. The functional and pharmacological analysis of chimeric $mGlu_{2}/_{1}$ receptors (Takahashi et al., 1993), $mGlu_{3}/_{1}$ receptors (Wroblewska et al., 1997), $mGlu_{4}/_{1}$ receptors (Tones et al., 1995) chimeras indicate that this large extracellular domain is involved in the selective recognition of agonists and competitive antagonists. A similar conclusion was obtained with chimeric receptors constructed with $mGlu_1$ receptors and the Drosophila $DmGlu_A$

receptor (Parmentier et al., 1998), as well as the $mGlu_1$ receptor and the Ca^{2+}-sensing receptor (Hammerland et al., 1999). Moreover, the production of the extracellular domain of $mGlu_1$ and $mGlu_4$ receptors as soluble proteins has been achieved and these domains were found to be sufficient for the binding of $mGlu_1$ and $mGlu_4$ receptor ligands, respectively (Okamoto et al., 1998; Han and Hampson, 1999). Similarly, $GABA_B$ ligands bind on the extracellular domain of the $GABA_B$ receptor subunit BR1 (Galvez et al., 1999; Malitschek et al., 1999).

In 1993, Patrick O'Hara and his colleagues, proposed the extracellular domain of mGlu receptors share structural similarity with some bacterial periplasmic binding proteins (O'Hara et al., 1993). A similar structure was also proposed for the agonist binding site of the ionotropic glutamate receptors, and this has been recently verified by resolving the crystal structure of this domain in the Glu_2 receptor subunit (Armstrong et al., 1998). In the case of mGlu receptors, this proposal is of course supported by the above mentioned experiments, but also confirmed by molecular modeling and mutagenesis studies of the $mGlu_1$ receptors (O'Hara et al., 1993; Costantino and Pellicciari, 1996), the $mGlu_2$ receptors (Yang et al., 1998) and the $GABA_B1$ receptor (Galvez et al., 1999) extracellular domains. According to this model, the binding domain of family 3 heptahelical receptors is constituted of two lobes interconnected by three linkers (Fig. 1). The agonist is supposed to bind on the larger lobe (lobe I on Fig. 1) within the cleft between the two lobes. By analogy with what has been observed with the bacterial periplasmic binding proteins (Quiocho, 1990), binding of an agonist is supposed to induce the closure of the two lobes, such that the ligand is then trapped in a cavity formed by these two lobes.

Homology modeling and mutagenesis experiments suggest that Ser165 and Thr188 of $mGlu_1$ receptors, two residues conserved in the entire mGlu receptor family including *Drosophila* and *Caenorhabditis elegans* mGlu receptors, play a critical role in glutamate binding (O'Hara et al., 1993). By analogy with what has been observed by X-ray crystallography for the binding of leucine in the bacterial protein leucine, isoleucine, valine binding protein, these two residues have been proposed to form hydrogen bonds with the α-carboxylic and α-amino groups, respectively. Recently, other residues involved in the binding of a group-II mGlu receptor ligand have been identified (Yang et al., 1998). These include Arg^{57} and Ser^{167} which are not conserved in the mGlu receptors from the other

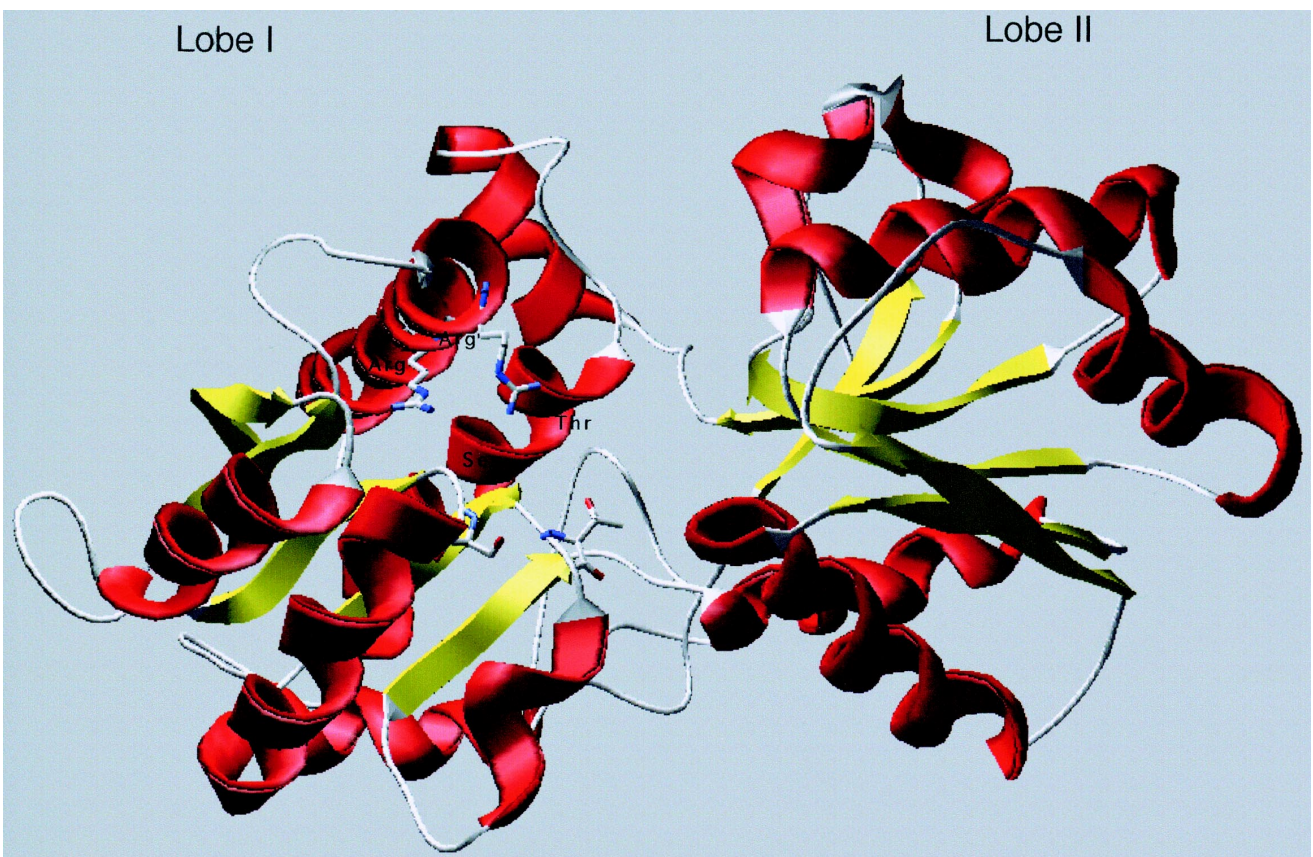


Fig. 1. Three dimensional model of the open state of the $mGlu_2$ receptor binding domain. This model has been generated according to the sequence alignment of the extracellular domain of mGlu receptors with periplasmic binding proteins as proposed by O'Hara et al. (1993) and using the program modeler (Molecular Simulation, San Diego, USA). The three large insertions not found in the bacterial periplasmic proteins have not been included in the model. Helices are indicated in red and sheets in yellow. Residues likely involved in the binding of glutamate are indicated.

groups and may therefore play a role in ligand recognition selectivity rather than in the direct interaction with glutamate.

2.2. The G-protein coupling domain

The molecular determinants of the interaction between the rhodopsin-like heptahelical receptors and the G-protein have been extensively studied (Bourne, 1997; Wess, 1997). It is assumed that the second and third intracellular loops of these receptors form a cavity where the last few carboxy-terminal residues of the G-protein α-subunit bind. Because the intracellular loops of family 3 receptors share no sequence similarity with those of the family 1 receptors, it was of interest to examine the molecular determinants of family 3 receptor G-protein coupling. The functional analysis of chimeric and mutated mGlu receptors generated using receptors with different G-protein coupling selectivity, revealed that the second intracellular loop plays a critical role for the recognition of the G-protein, whereas the other intracellular domains mostly control the coupling efficacy (Pin et al., 1994; Gomeza et al., 1996a; Francesconi and Duvoisin, 1998) (Fig. 2). On the G-protein side, the extreme carboxy-terminus of the α-subunit

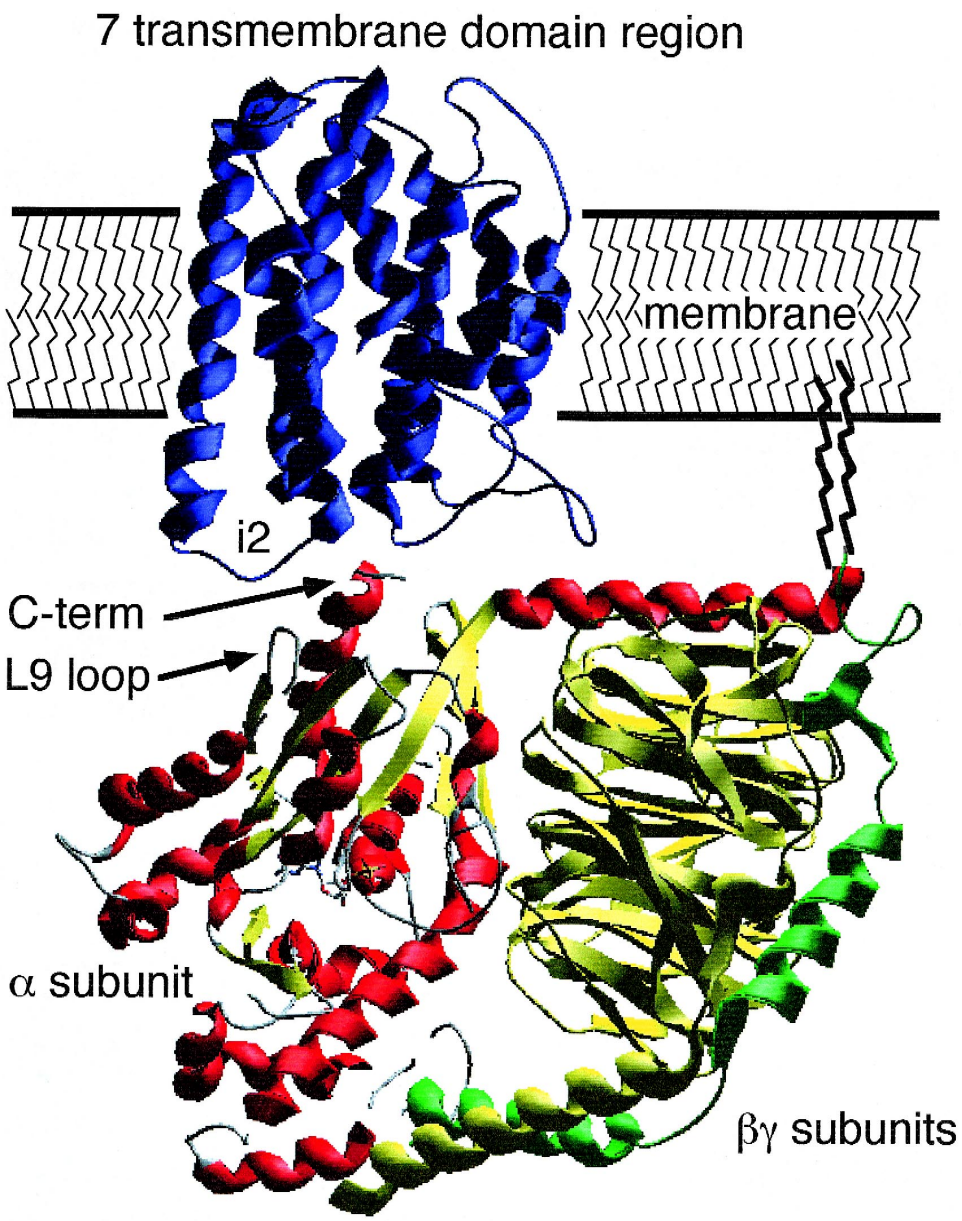


Fig. 2. Three dimensional view of the seventh transmembrane domain region of mGlu receptors and its association with the heterotrimeric G-protein. The α subunit is shown in red (helices) and yellow (sheets), the β subunit is in yellow, and the γ subunit in green. The regions involved in the specificity of the receptor-G-protein interaction are indicated: the second intracellular loop of the receptor (i2), the carboxyl-terminal end (C-term) and the L9 loop of the G-protein α-subunit.

plays a critical role for the recognition of family 3 receptors (Gomeza et al., 1996b; Blahos et al., 1998), as previously reported for family 1 receptors (Bourne, 1997; Wess, 1997). We therefore proposed that the carboxy-terminus of the G-protein α-subunit is also interacting in a cavity formed by the second and third intracellular loops of family 3 receptors (Fig. 2). However, we hypothesized the second intracellular loop of these receptors plays a role equivalent to that of the third intracellular loop of the rhodopsin-like receptors in G-protein activation.

Aside from a better understanding on how a seventh transmembrane domain protein interacts and activates the heterotrimeric G-proteins, such a study has important consequences for the development of functional tests for the different mGlu receptor subtypes. A chimeric G-protein α-subunit corresponding to the phospholipase C-activating $G_{\alpha q}$ subunit with its last 5–9 carboxy-terminal residues replaced by those of $G_{\alpha o}$ or $G_{\alpha i}$, allows the coupling of group-II or group-III mGlu receptors to phospholipase C and the release of Ca^{2+} from the intracellular stores. Such a transduction cascade is easier to measure than the inhibition of the forskolin-induced cAMP production, and has been successfully used by our group to determine the pharmacological profile of many group-II and group-III mGlu receptors (Gomeza et al., 1996b; Parmentier et al., 1996; Acher et al., 1997; Brabet et al., 1998; Pellicciari et al., 1998; Tellier et al., 1998). Such chimeric G-proteins appear therefore as an excellent tool for the development of high-throughput screening tests for group-II and group-III mGlu receptor ligands.

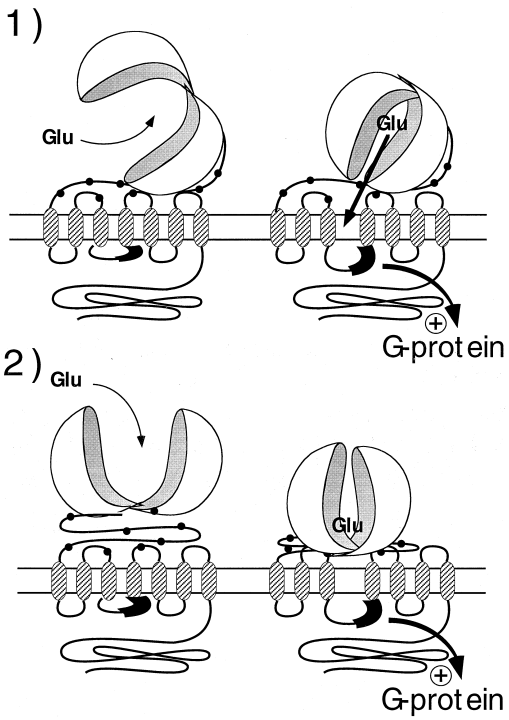


Fig. 3. Two hypotheses that can be proposed to explain how the binding of the agonist in the large extracellular domain of mGlu receptors leads to the activation of the transmembrane region. The first hypothesis is based on our knowledge of the mechanism of action of bacterial periplasmic proteins, and on our knowledge of the mechanism of action of family-1 receptors. The second more probable hypothesis is that the liganded closed form of the extracellular domain activates the transmembrane region by interacting with the extracellular loops.

2.3. Possible mechanisms for receptor activation

Because the ligand binding domain of family 3 receptors likely has a structure similar to that of periplasmic binding proteins, this domain of the receptor may function similarly as these bacterial proteins. These proteins act as transporters in bacteria. Upon ligand binding, they close like a clam shell, and then deliver their ligand to another transmembrane protein that is responsible for the transport of the ligand through the bacterial inner membrane (Quiocho, 1990). Accordingly, two main hypotheses can be proposed for the mechanism of activation of family 3 receptors (Fig. 3). In a first hypothesis, the binding of the agonist in the extracellular domain induces the closure of the two lobes and then the agonist is delivered in a second site located in the seventh transmembrane domain region. The agonist-binding in this site will stabilize the active conformation of the seventh transmembrane domain region, like most agonists of the family 1 receptors do. Although this hypothesis will fit with our understanding of the mechanism of action of bacterial periplasmic binding proteins and of family 1 receptors, one may assume the seventh transmembrane domain region plays a role in the activity of mGlu receptor ligands. No such observation has been reported yet despite the large number of chimeric receptors already analyzed. Moreover, the functional coupling of chimeric $mGlu_1/Ca$ and $Ca/mGlu_1$ receptors to G-proteins upon glutamate and Ca^{2+} application, respectively, does not support this first hypothesis (Hammerland et al., 1999). A second hypothesis would be that the closed form of the ligand binding domain is viewed as the activator of the seventh transmembrane domains region. Accordingly, the stabilization of the closed form of the binding domain by an agonist will lead to receptor activation (Fig. 3).

2.4. Is the dimerization process necessary for mGlu receptor function?

The classical view of heptahelical receptor/G-protein coupling stoichiometry is one receptor for one G-protein. However, the functional analysis of chimeric and mutated receptors revealed that they can dimerize (Maggio et al., 1993; Monnot et al., 1996) possibly via a coiled-coil interaction of their sixth transmembrane domain (Hebert et al., 1996). This dimerization process has been proposed to be important for the G-protein activation (Hebert et al., 1996). The importance of receptor dimerization has recently been documented in the family 3 heptahelical receptors. The mGlu receptors and the Ca^{2+}-sensing receptor

are homodimers, disulfide linked at the level of their large extracellular domain (within the first 100 residues) (Romano et al., 1996; Bai et al., 1998; Ward et al., 1998) (Fig. 4). Even when produced as a soluble protein the extracellular domain of the $mGlu_1$ receptor forms disulfide-linked dimers (Okamoto et al., 1998). Although dithiothreitol treatment of cells expressing $mGlu_5$ receptors suppresses receptor function, it is not known whether this results from the breakdown of the disulfide bond(s) involved in the dimerization, or to a reduction of any other disulfide bonds likely important for receptor activation. Recently, two cysteine residues in the extracellular domain of the Ca^{2+}-sensing receptor have been shown to affect the formation of dithiothreitol-sensitive dimers, and to prevent Ca^{2+} from activating the receptor (Fan et al., 1998). These two cysteines are conserved in all mGlu receptors and vomeronasal receptors, but not in the $GABA_B$ receptors which do not form disulfide linked dimers (see bellow). These results suggest that the covalent dimerization is necessary for the activation of these receptors by an agonist. However, a functional activation of a mutated Ca^{2+}-sensing receptor devoid of its large extracellular domain has been achieved with Gd^{3+}, indicating that the covalent dimerization of the extracellular domain is not necessary for the functional activation of the G-protein by the seventh transmembrane domains region (Hammerland et al., 1999). This does not exclude the requirement of a dimer of the seventh transmembrane domain region since this domain of the Ca^{2+}-sensing receptor has been shown to dimerize on its own, independently of the extracellular domain, probably like the other seventh transmembrane domains receptors (Bai et al., 1998).

Very recently, the $GABA_B$ receptor has been shown to be a heterodimer (or heteromultimer) (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999) constituted of two 'subunits' sharing sequence similarity: GABA-BR1 and GABA-BR2. None of these 'subunits' gave rise to a fully functional receptor when expressed alone, but co-expression of both 'subunits' gave rise to a $GABA_B$ receptor efficiently coupled to G-proteins. In that particular case, the oligomers are not linked by a disulfide bond in the extracellular domain, but by a coiled coil interaction of their carboxy-terminal intracellular tails (White et al., 1998; Kuner et al., 1999). This observation further indicates that seventh transmembrane domains receptors function as dimers (or multimers).

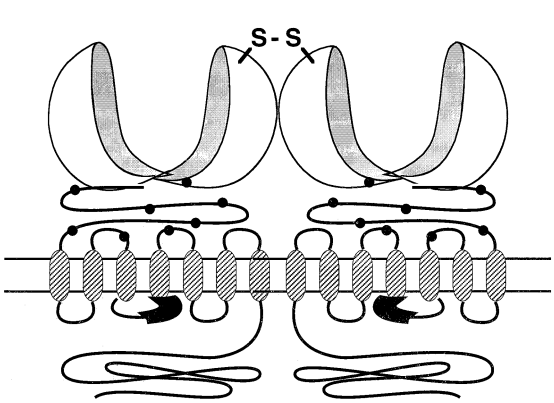


Fig. 4. mGlu Receptors as well as the Ca^{2+}-sensing receptor form homodimers linked by disulfide bonds within their extracellular domain.

2.5. The intracellular carboxy-terminus

Many roles of the carboxy-terminus of heptahelical receptors have been described, ranging from the control of G-protein coupling, desensitization and down-regulation phenomena, to the interaction with specific regulatory and effector proteins (for a recent review, see Bockaert and Pin, 1999). In the case of mGlu receptors, very few studies were dedicated to the determination of the specific roles of their carboxy-terminal intracellular domain.

The phospholipase C-coupled mGlu receptors, $mGlu_{1a}$ and both $mGlu_5$ receptor variants have a surprisingly long carboxy-terminus of more than 350 residues, and all have a high basal, i.e., agonist-independent-activity (Joly et al., 1995; Prézeau et al., 1996). In contrast, no such a basal activity can be detected with the short $mGlu_1$ receptor splice variants, $mGlu_{1b,c,d}$ receptors that lack the last 313 residues specific for $mGlu_{1a}$ receptor. Mutagenesis experiments demonstrated that the absence of basal activity of these short receptors resulted from the inhibitory action of a cluster of four basic residues located close to their extreme carboxy terminus (Mary et al., 1998). Since this cluster of basic residues is also found in the long $mGlu_{1a}$ receptor isoform, we proposed the long carboxy-terminal sequence of $mGlu_{1a}$ receptor prevents the action of this short inhibitory element. This shows the carboxy-terminus of $mGlu_1$ receptors controls the G-protein coupling efficacy.

The carboxy-terminus of mGlu receptors is likely involved is their desensitization. The protein kinase C-dependent phosphorylation of the intracellular regions of the phospholipase C-coupled $mGlu_{1a}$ receptors has been described (Alaluf et al., 1995). This is likely to be also the case for the $mGlu_5$ receptor for which specific serine and threonine residues are involved in the agonist-dependent desensitization (Gereau and Heinemann, 1998). The phosphorylation of a single threonine residue in the carboxy-terminus of the $mGlu_5$ receptor has also been shown to play a key role in the generation of Ca^{2+} oscillations by $mGlu_5$ receptors (Kawabata et al., 1996). This type of Ca^{2+} signal is not generated by $mGlu_1$ receptors in which this threonine residue is replaced by an aspartate. The phosphorylation of the carboxy-terminal domain of $mGlu_5$ receptors has also been reported to control its association with calmodulin (Minakami et al., 1997). However, the functional role of the interaction is not known.

Recently, the protein Homer1 (also called Vesl1) has been shown to interact with the consensus sequence ele-

ment PPxxFR which is found in the carboxy-terminal end of the $mGlu_{1a}$ and $mGlu_5$ receptors, but also in the inositol triphosphate (IP_3) receptor (Brakeman et al., 1997; Kato et al., 1998; Tu et al., 1998; Xiao et al., 1998). Homer1 exists as three distinct splice variants, Homer1a, Homer1b and Homer1c, which all contain an EVH1 (EVH for ena-Vasp homology) domain able to interact with the mGlu receptors and the IP_3 receptors. However, only Homer1b and Homer1c possess an additional carboxy-terminal helical domain allowing dimerization of the homer protein via a coiled-coil interaction. Accordingly, Homer1b or Homer1c can attach $mGlu_{1a}$ or $mGlu_5$ receptors to the IP_3 receptor, allowing a more efficient coupling between the receptor and the release of Ca^{2+} from the intracellular stores (Tu et al., 1998). Since Homer1a, which does not dimerize, competes with Homer1b or Homer1c for the binding to mGlu receptors and IP_3 receptors, it may act as a dominant negative protein preventing the anchoring of the mGlu receptor to the IP_3 receptor (Tu et al., 1998). Interestingly, Homer1a is highly expressed in neurons under over-excitatory conditions (Brakeman et al., 1997; Kato et al., 1998). Additional Homer proteins which also interact with $mGlu_{1a}$ and $mGlu_5$ receptors have been identified (Homer2 and Homer3) (Kato et al., 1998; Xiao et al., 1998). Their role remains to be analyzed.

Another putative function of the carboxy-terminal intracellular domains of mGlu receptors is their targeting to specific compartments of the neuron. Each mGlu receptor subtype has been shown to have a very restricted localization in specific compartments of the neuron. For example, $mGlu_7$ receptor has been shown to be restricted to the active zone in presynaptic terminals (Shigemoto et al., 1996). Using the adenovirus expression system to transfect hippocampal neurons differentiated in primary culture, it has been shown that the carboxy-terminus of the $mGlu_7$ receptor plays a critical role in its specific targeting to the axonal compartment (Stowell and Craig, 1999).

3. New development of selective mGlu receptor ligands

Aside from glutamate, additional endogenous molecules display agonist activities at various mGlu receptor subtypes. These include the sulfur containing amino acids cysteate, cysteine sulfinate, homocysteate, homocysteine sulfinate (Thomsen et al., 1993; Johansen et al., 1995; Kingston et al., 1998a), and L-serine-*O*-phosphate the later being more potent than glutamate at most group-III mGlu receptors (Table 2). Recently, the dipeptide *N*-acetyl-aspartyl-glutamate (NAAG), which has long been proposed as an endogenous neurotransmitter, has been shown to selectively activate group-II mGlu receptors, with a higher potency on $mGlu_3$ receptors than on $mGlu_2$ receptors (Wroblewska et al., 1997; Schaffhauser et al., 1998).

The first specific metabotropic glutamate receptor agonist and antagonist, 1*S*,3*R*-1-amino-1,3-cyclopentanedicarboxylate (1*S*,3*R*-ACPD) and *S*-α-methyl-4-carboxyphenylglycine (MCPG), respectively, have been very useful for the characterization of some physiological roles of these receptors (Conn and Pin, 1997). However, these compounds are not selective (1*S*,3*R*-ACPD act on both group-I and group-II mGlu receptors, and MCPG is an antagonist at many mGlu receptor subtypes), and have a low affinity. Care should also be taken when analyzing data obtained with the so-called selective mGlu receptor agonists described bellow, since their activity at ionotropic glutamate receptors has not always been carefully studied (Contractor et al., 1998). Of interest, compounds acting as agonist or antagonist on all mGlu receptor subtypes with a relatively similar affinity have been discovered recently: 2-aminobicyclo[2.1.1]hexane-2,5-dicarboxylate-I (ABHxD-I) (Kozikowski et al., 1998) and (1*R*,3*R*,4*S*) 1-amino cyclopentane-1,3,4-tricarboxylate (ACPT-II) (Acher et al., 1997), respectively. Such compounds may be useful for the demonstration that an mGlu receptor, whatever subtype, is involved in a physiological process. However, high affinity subtype selective compounds are absolutely necessary for the dissection of the multiple physiological roles of each mGlu receptors, both in in vitro and more importantly, in in vivo preparations.

3.1. The group-I selective ligands

Although the $mGlu_1$ receptor, a group-I receptor, has been the first to be cloned, and as such has been the subject of intense research, very few group-I selective compounds are available. The most potent group-I agonist remains quisqualate, which is 10 to 100 fold more potent on these receptors than on the AMPA receptors. The most selective group-I agonist is 3,5-dihydroxyphenylglycine (3,5-DHPG), although it has a lower potency than quisqualate (Conn and Pin, 1997) and also acts on NMDA receptors at high concentration (Contractor et al., 1998). More recently, CHPG has been proposed as a selective $mGlu_5$ receptor agonist (Doherty et al., 1997). However, its very low potency prevents its careful characterization on the other mGlu receptor subtypes.

Most group-I competitive antagonists characterized so far are rather specific for $mGlu_1$ receptor. The most potent $mGlu_1$ receptor antagonists are 4-carboxy-3-hydroxyphenylglycine (4C3HPG), 2-(3′-carboxybicyclo[1.1.1]pentyl)-glycine (CBPG) (Pellicciari et al., 1996) and (*S*)-2-methyl-4-carboxyphenylglycine (LY367385) (Clark et al., 1997). These are either inactive or act as partial agonists on $mGlu_5$ receptor (Conn and Pin, 1997). Actually, the only commercially available $mGlu_5$ receptor antagonist is the low affinity non-selective MCPG. The Eli Lilly research team recently reported the characterization of a new group-I antagonist, α-thioxanthylmethyl-3-carboxycyclobutylglycine (LY393675) which has a similar high potency on both $mGlu_1$ and $mGlu_5$ receptors (Baker et al., 1998). Few years ago, 7-(hydroxyimino)cyclopopa[*b*]chromen-1a-

Table 2
Activities of several compounds on cloned mGlu receptors

	mGluR1a	mGluR5a	mGluR2	mGluR3	mGluR4a	mGluR6	mGluR7a	mGluR8a
Endogenous agonists								
Glutamate	9–13	3–10	4–20	4–5	3–20	16	1000	2.5–11
Aspartate	–	–	69	49	n.e.	–	n.e.	47
L-Cysteate	84	31	–	–	–	–	–	–
L-Cysteine sulfinate	58	54	–	–	n.e.	–	–	–
L-Homocysteate	n.e.	n.e.	–	–	49–56	–	–	–
L-Homocysteine sulfinate	39–300	45	–	–	–	–	–	–
L-Serine-*O*-phosphate	n.e.	n.e.	ant. (23)	ant. (53)	1–4	2.7	31–1200	0.3–1.8
NAAG	n.e.	n.e.	134–1000	~ 10	n.e.	n.e	–	–
Agonists								
S-2-aminoadipate	n.e.	–	35	–	n.e.	140	–	–
ABHxD-l	1.6	0.7	0.3	2	23	5	–	–
1*S*,3*R*-ACPD	5–80	5–40	7–18	6–17	100–1000	300	n.e.	45–166
1*S*,3*S*-ACPD	> 100	> 300	4–13	11–30	1000	–	–	6–243
ACPT-I	n.e.	–	n.e.	–	7.2	–	–	13
(+)ACPT-III	ant.	–	ant.	–	8.8	–	–	7
t-ADA	190	30	> 1000	–	n.e.	–	–	–
L-AP4	n.e.	n.e.	n.e.	n.e.	0.2–1.2	0.9	160–500	0.06–0.60
2*R*,4*R*-APDC	> 100	> 100	0.3–3.0	0.4	n.e.	> 100	n.e.	> 100
CBAP5	–	–	–	–	4–33	–	–	15
L-CCG-I	2–50	3–17	0.1–0.7	0.3–1.0	4–50	6	50–200	0.3–3.5
4C3HPG	ant.	> 200	20–50	–	n.e.	–	–	126
CPrAP4	–	–	–	–	0.6	–	–	–
CPeAP4					26–115			63
DCG-IV	ant.	–	0.1–0.3	0.1–0.2	ant.	ant.	ant.	ant.
3,5-DHPG	6–30	2–10	n.e.	n.e.	n.e.	–	n.e.	n.e.
γ-Carboxyl-L-glutamate	n.e.	n.e.	ant. (100)	ant. (300)	Pag. 84	59	ant. (120)	ant. (300)
S-HomoQuis	ant. (184)	36	23	–	n.e.	–	–	–
S-HomoAMPA	n.e.	n.e.	n.e.	n.e.	n.e.	58	n.e.	–
3-Hydroxyphenylglycine	68–100	14–35	n.e.	–	n.e.	–	–	–
Ibotenate	2–60	2–17	35–250	10–15	50–1000	n.e.	–	> 300
LY354740	> 100	> 100	0.005	0.024	> 100	–	> 100	36
LY379268	> 100	> 100	0.00032	0.00015	> 100	–	> 100	1.69
4-Phosphonophenylglycine	> 200	> 200	> 200	> 200	5.2	4.7	185	0.2
Quisqualate	0.1–3.0	0.03–0.3	100–1000	27–40	100–1000	n.e.	n.e.	719
Antagonists								
AIDA	7–300	–	n.e.	–	n.e.	–	–	–
ACPT-II	115	–	88	–	77–125	–	–	123
L-AP3	> 1000	> 1000	[17]	[125]	n.e.	–	n.e.	–
APICA	n.e.	n.e.	30	–	–	n.e.	–	–
4-Bromo-homoibotenate	160	230	n.e.	–	n.e.	–	–	–
CBPG	25–32	PAg.	n.e.	–	n.e.	–	–	–
4C3HPG	10–40	P.Ag.	Ag.	–	n.e.	–	–	–
3C4HPG	300–400	–	Ag.	–	n.e.	–	–	–
CPCCOEt	10–35	n.e.	n.e.	–	n.e.	–	n.e.	n.e.
4CPG	15–65	> 500	Ag.	–	n.e.	–	–	–
DCG-IV	389	630	Ag.	Ag.	22	40	25–40	15–32
EGlu	–	–	[42]	–	> 1000	–	–	689
LY341495	7	8	0.021	0.014	22	–	0.99	0.17
LY307452	n.e.	n.e.	18–50	30	n.e.	–	n.e.	n.e.
LY367385	8	> 100	–	–	–	–	–	–
LY393675	0.35	0.47	–	–	–	–	–	–
MAP4	n.e.	–	500	–	90–190	–	–	25–105
MCCG-I	n.e.	–	25–84		n.e.	–	–	> 300
MCPG	40–200	> 200	300–1000	> 1000	n.e.	–	n.e.	> 300
MPPG	≫ 1000	n.e.	11–300	–	54–500	480	300	20–50
MPEP	> 10	0.036	> 10	–	> 10	–	> 10	> 10
MSPG	n.e.	–	250	–	≫ 1000	–	–	476
MTPG	> 300	n.e.	450	–	n.e.	–	–	558
NPS2390	0.005	–	–	–	–	–	–	–
PCCG-IV	n.e.	n.e.	8	–	Ag.	–	–	–

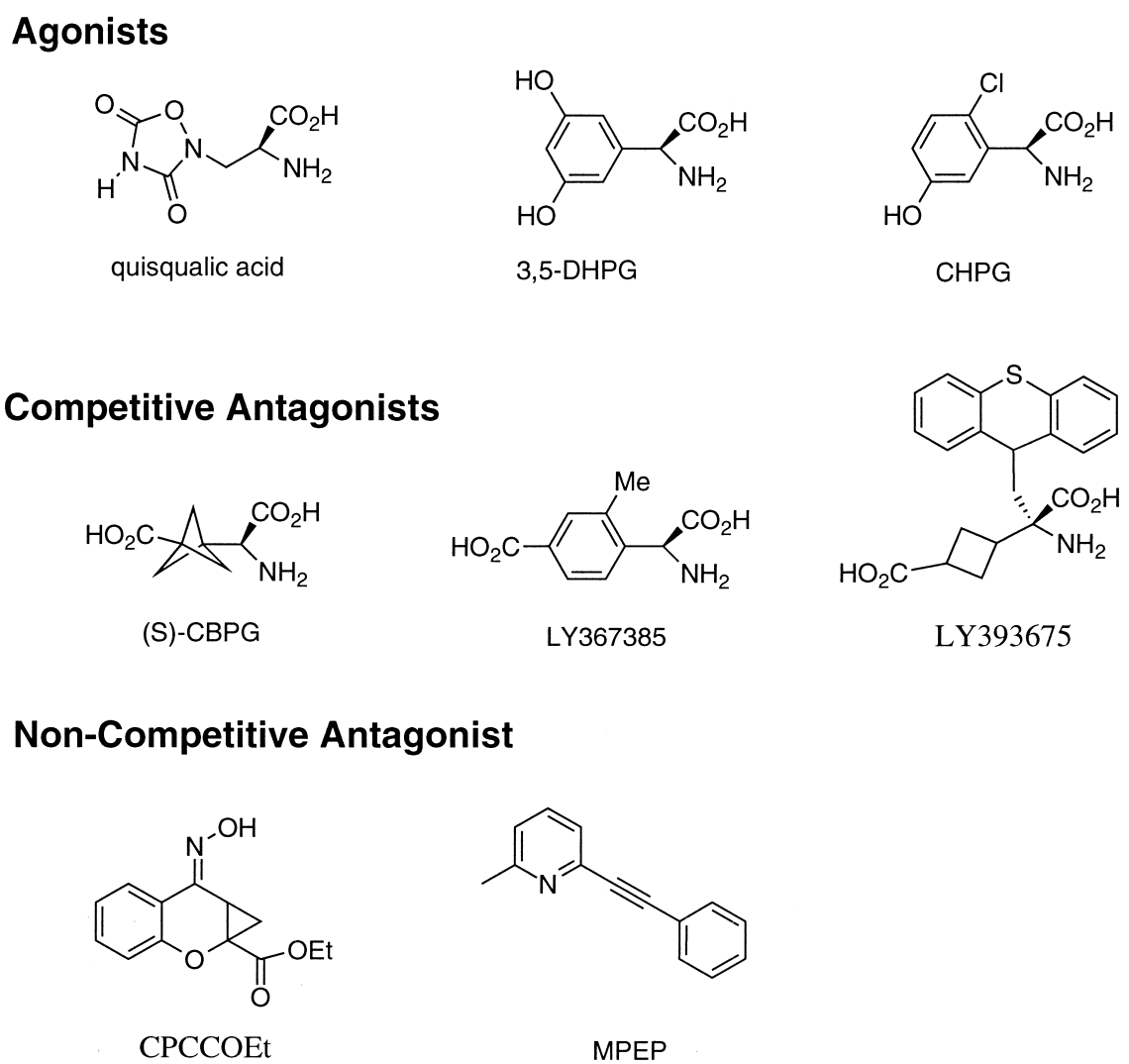


Fig. 5. Structure of group-I mGlu receptor ligands.

carboxylate ethyl ester (CPCCOEt) has been reported to be a potent $mGlu_1$ receptor antagonist, though it has no amino acid like structure (Annoura et al., 1996). Recently, this compound was shown to be selective for, and to act as a non-competitive antagonist unable to displace [^{3}H]glutamate or [^{3}H]quisqualate binding on $mGlu_1$ receptors (Her-

Notes to Table 2:
Data are EC_{50} (for agonist activities) or IC_{50} (for antagonist activities or displacement of bound radioligand) expressed in μM.
Values are taken form the references as previously reported (Conn and Pin, 1997). More recent data were taken from the following references (Acher et al., 1997; Ahmadian et al., 1997; Bräuner-Osborne et al., 1997; Clark et al., 1997; Flor et al., 1997; Ma et al., 1997; Schoepp et al., 1997; Tückmantel et al., 1997; Wroblewska et al., 1997; Baker et al., 1998; Brabet et al., 1998; Brauner-Osborne and Krogsgaard-Larsen, 1998; Brauner-Osborne et al., 1998; Corti et al., 1998; Escribano et al., 1998; Kingston et al., 1998a; Kingston et al., 1998b; Kozikowski et al., 1998; Litschig et al., 1998; Monn et al., 1998; Parmentier et al., 1998; Pellicciari et al., 1998; Valli et al., 1998; Wu et al., 1998). So far, no pharmacological differences have been reported between the human and rat receptors.
The values obtained with receptors from both species were therefore included.
Values in square brackets correspond to IC_{50} value determined in binding experiments.
n.e.: No effect.
–: Not tested.
ant.: Antagonist activity.
Ag.: Agonist activity.
The abbreviations used in this table and not defined in the text are as follow: *t*-ADA: *trans*-azetidine-2,4-dicarboxylate; AIDA: 1-aminoindan-1,5-dicarboxylate; AP3: 2-amino-3-phosphonopropionate; 3C4HPG: 3-carboxy-4-hydroxyphenylglycine; CPeAP4: 1-amino-*cis*-3-phosphonocyclopentanecarboxylate; 4CPG: 4-carboxyphenylglycine; CPrAP4: cyclopropyl-AP4 (2-amino-2,3-methano-4-phosphonobutanoate); 3HPG: 3-hydroxyphenylglycine; MSPG: α-methyl-4-sulfonophenylglycine; MTPG: α-methyl-4-tetrazoylphenylglycine.

mans et al., 1998; Litschig et al., 1998; Okamoto et al., 1998). These data indicated that CPCCOEt interacts at a site different from the agonist binding site. As described bellow, this site has been mapped within the seventh transmembrane domain region of the $mGlu_1$ receptor (Litschig et al., 1998). Recently, a new series of highly selective, non-competitive and systemically active $mGlu_5$ receptor antagonists has been reported (Gasparini et al., 1999b; Varney et al., 1999), the more potent compound being 2-methyl-6-(phenylethynyl)pyridine (MPEP) (IC_{50} 36 nM) (Fig. 5 and Table 2). An even more potent non-competitive mGlu receptor antagonist acting at both $mGlu_1$ and $mGlu_5$ receptors has also been described, NPS 2390 (Van Wagenen et al., 1998).

3.2. The group-II selective ligands

New highly potent and selective agonists and antagonists have been characterized for group-II mGlu receptors. The first selective group-II agonist identified was 1*S*,3*S*-ACPD, followed by 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC). (2*S*,1′*S*,2′*S*)-2-(carboxycyc-

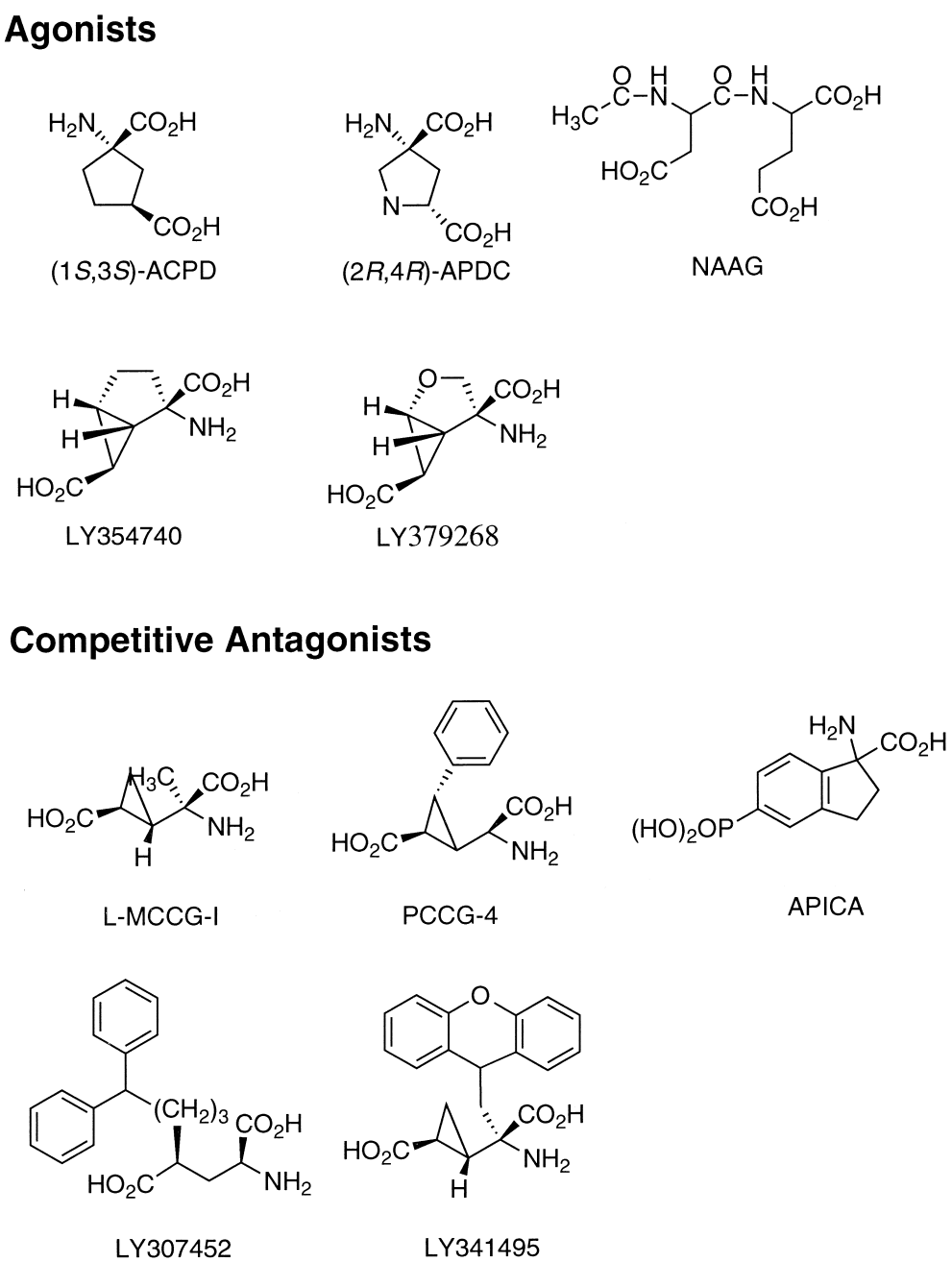


Fig. 6. Structure of group-II mGlu receptor ligands.

lopropyl)glycine (L-CCG-I) and (2*S*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) have also been proposed to be selective group-II agonists (Fig. 6). However, L-CCG-I is active on all mGlu receptors subtypes in the micromolar range, and DCG-IV is the most potent group-III antagonist known so far (Brabet et al., 1998). The first very potent agonist synthesized was (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0.]hexane-2,6-dicarboxylate (LY354740) (Schoepp et al., 1997), and its even more potent derivative 1*R*,4*R*,5*S*,6*R*-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) (Monn et al., 1998), both being active in the nanomolar range. Of great interest, these molecules pass the blood brain barrier and can therefore be used to analyze the physiological role of group-II mGlu receptors in in vivo situations, allowing the identification of putative therapeutic application of such drugs (Helton et al., 1997; Bond et al., 1998; Helton et al., 1998; Konieczny et al., 1998; Moghaddam and Adams, 1998). Although the pharmacological profiles of $mGlu_2$ and $mGlu_3$ receptors are very similar, *N*-acetyl-aspartyl-glutamate has been shown to be more potent on $mGlu_3$ receptors than on $mGlu_2$ receptors (Wroblewska et al., 1997; Schaffhauser et al., 1998), indicating that it will be possible to develop selective drugs acting on only one of these group-II receptors.

The first selective group-II antagonists identified were α-methyl-L-CCG-I (MCCG-I) (Conn and Pin, 1997) and the phenyl derivative of CCG-I, (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCG-IV) (Thomsen et al., 1996). (2*S*)-α-ethylglutamate (EGlu), has also been proposed as a new group-II/III antagonist, however, its action on cloned mGlu receptors has not been reported yet (Jane et al., 1996; Cartmell et al., 1998) (Table 1). A rigid analog of the phosphonophenylglycine, 1-amino-5-phosphonoindan-1-carboxylate (APICA), has recently been shown to be a specific group-II mGlu receptor antagonist although with a low affinity (Ma et al., 1997). Very potent and competitive group-II antagonists have been described: (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioate (LY307452) (Wermuth et al., 1996) and 2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoate (LY341495) (Kingston et al., 1998b). This latter compound is also antagonizing group-I mGlu receptors at higher concentrations (Kingston et al., 1998b). So far, no

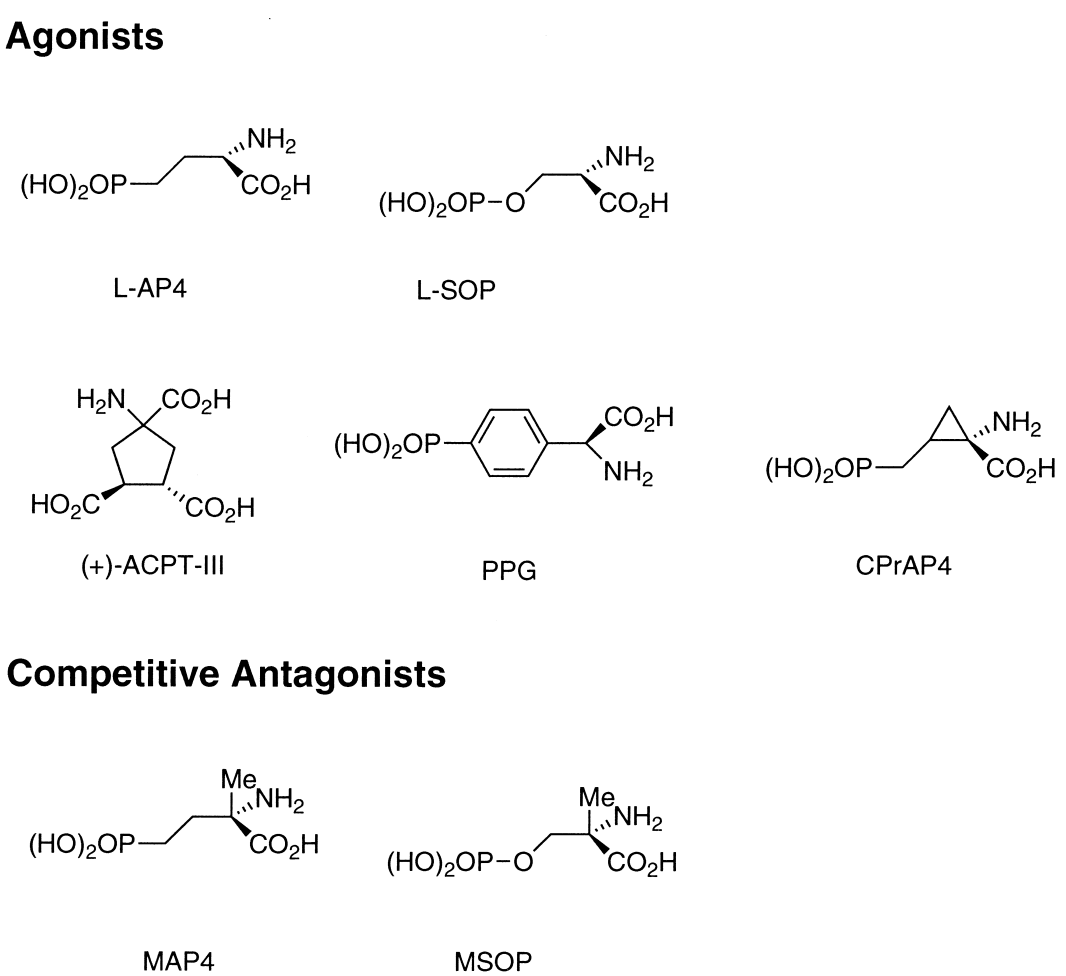


Fig. 7. Structure of group-III mGlu receptor ligands.

antagonist specific for either $mGlu_2$ or $mGlu_3$ receptors have been reported.

The very high potency of some group-II agonists and antagonists offer good possibilities for the development of highly selective radioligands. Within the last year, [^{3}H]DCG-IV (Cartmell et al., 1998), [^{3}H]LY354740 (Schaffhauser et al., 1998) and [^{3}H]LY341495 (Ornstein et al., 1998) have been reported a good radioligand for group-II receptors in native tissue or in heterologous expression systems.

3.3. The group-III selective ligands

Group-III mGlu receptors mainly act as glutamatergic auto-receptors, and as such group-III mGlu receptor agonists are expected to have multiple therapeutic applications by inhibiting the glutamatergic transmission (Fig. 7). It is therefore very surprising that the group-III mGlu receptor pharmacology remains so poor. The most potent group-III agonists are still L-1-amino-4-phosphosnobutyrate (L-AP4) and L-serine-*O*-phosphate which unfortunately also act on other systems such as on a glutamate transporter (Pin et al., 1984; Conn and Pin, 1997). Only recently have a few new selective group-III mGlu receptor agonists been described: (1*S*,3*R*,4*R*)-1-aminocyclopentane-1,3,4-tricarboxylate (ACPT-I), (3*S*,4*S*) 1-aminocyclopentane-1,3,4-tricarboxylate ((+)-ACPT-III) (Acher et al., 1997) and 4-phosphonophenylglycine (Gasparini et al., 1999a). However, their activity has not yet been analyzed on all four group-III mGlu receptors. CPrAP4 and 1-amino-3-(phosphonomethylene)cyclobutane (CBAP5) have also been reported as potent $mGlu_4$ receptor agonists, but their effects on other receptor subtypes remain to be studied (Johansen et al., 1995).

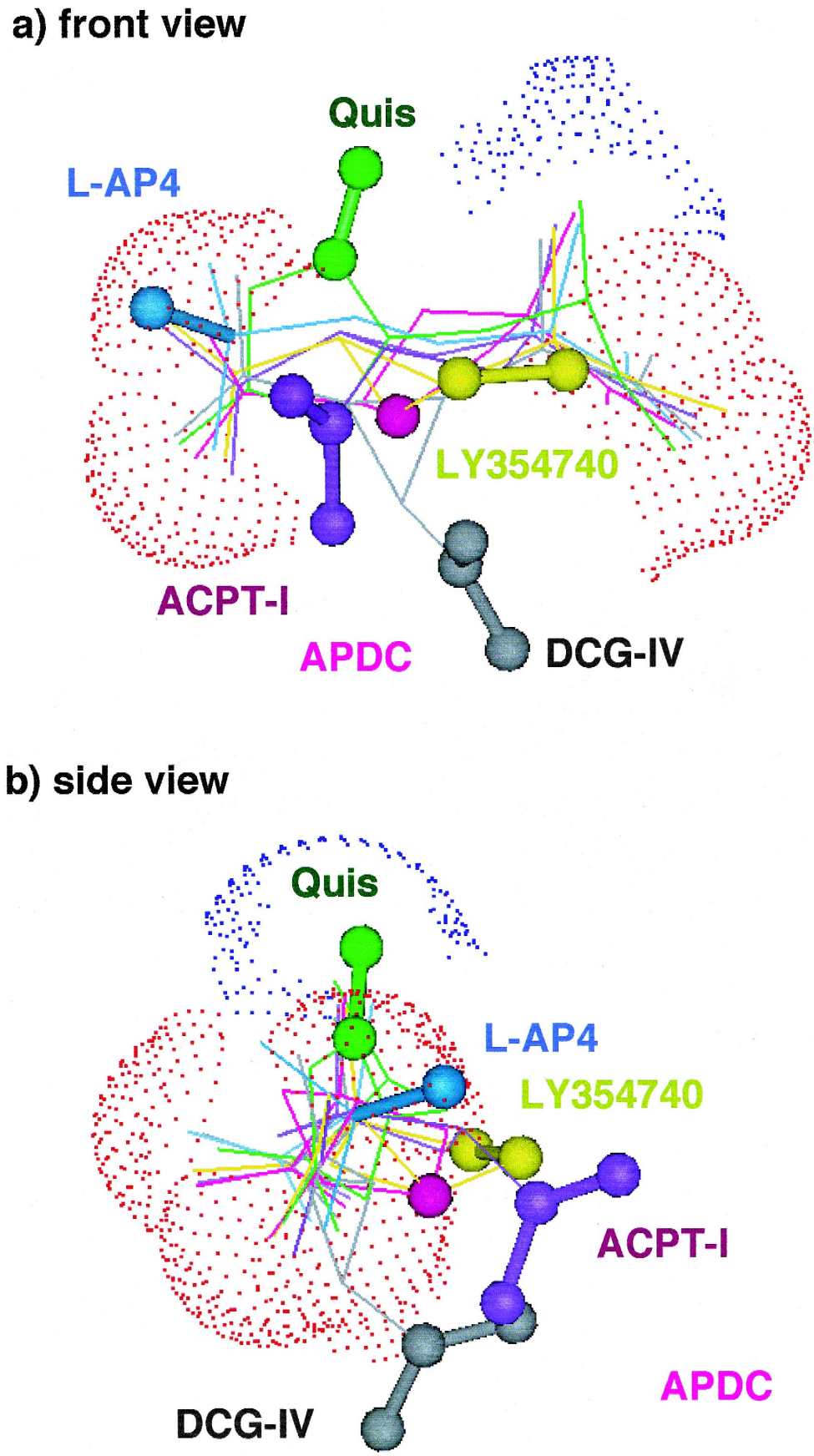
The first selective group-III antagonist described was α-methyl-AP4 (MAP4). α-Methyl-4-phosphonophenylglycine (MPPG) has also been proposed as a potent group-III antagonist. However, when tested on cloned receptors expressed in heterologous systems, it was found to be also active on group-II mGlu receptors (Conn and Pin, 1997) (Table 2). α-Cyclopropyl-4-phosphonophenylglycine (CPPG) has been reported as a specific group-III antagonist (Jane et al., 1996), but its action on cloned mGlu receptors has not been reported yet. Recently, we reported that DCG-IV is a potent group-III antagonist (Brabet et al., 1998), although acting at higher concentrations than those required to activate group-II mGlu receptors. This information may be useful for the development of selective and more potent group-III antagonists.

4. Pharmacophore models of mGlu receptors reveal new features for the development of new ligands

A three-dimensional structure based on homology with the bacterial periplasmic binding proteins (see above) has been proposed for the mGlu receptors binding domain. However, the low sequence similarity between these proteins prevent the generation of a precise enough model allowing an accurate docking of mGlu receptor ligands. Alternately, pharmacophore models afford an insight into the glutamate bound conformation and into the topology of its protein environment. Such information would greatly help the generation of better model of the binding pocket of these receptors. With the increasing number of agonists assayed at some mGlu receptors, and especially the characterization of rigid molecules such as ABHxD (Kozikowski et al., 1998) and ABHD (Tellier et al., 1998) compounds, it became possible to construct accurate pharmacophore models using molecular modeling. For $mGlu_1$, $mGlu_2$ and $mGlu_4$ receptors which are representatives of group-I, group-II and group-III mGlu receptors, respectively, a pharmacophore model has been established (Costantino et al., 1993; Bessis et al., 1999; Jullian et al., 1999). They show that in all three cases, glutamate would be recognized in an extended conformation, which is characterized by large distances between the two acidic groups or between the α-amino and γ-acidic groups. Additional selective interactions have been identified and can be described with a few selective ligands as displayed in Fig. 8. According to the model, quisqualic acid would define a specific $mGlu_1$ receptor hydrophilic site through its additional carbonyl group. LY354740 and (2*R*,4*R*)-APDC would delineate two $mGlu_2$ receptor selective regions, where a large hydrophobic substituent would increase affinity or a positive charge would be accepted, respectively. L-AP4 and ACPT-I in turn, would define two specific hydrophilic sites in the $mGlu_4$ receptor binding pocket with the additional phosphonic oxygen atom or the third carboxylic function, respectively. Finally, DCG-IV would disclose a region where the third carboxylic group would prevent binding at $mGlu_1$ receptors, allow potent activity at $mGlu_2$ receptors and prevent the activation process at $mGlu_4$ receptors (therefore explaining the antagonist action of this compound at this receptor subtype).

Interestingly all chemical groups generating selectivity, are localized in the same front face of Fig. 8A, as it can be seen in Fig. 8B when the central atom of the distal acidic group and the α-carbon atom of ligands are eclipsed (side view). In the upper part of that face is found the quisqualate

Fig. 8. Superposition of selective ligands in their conformation adopted in the respective pharmacophore models: quis ($mGlu_1$), LY354740 and (2*R*,4*R*)-APDC ($mGlu_2$), L-AP4 and ACPT-I ($mGlu_4$), and DCG-IV (all three mGlu receptors). The alpha-nitrogen atom and the alpha- and gamma-acidic oxygen atoms common to all compounds have been superimposed. They are displayed as Connolly solvent accessible surfaces: blue dot spheres for nitrogen, red ones for oxygens. Selective chemical groups are shown with ball and sticks representation. (A) Front view; (B) side view.


a) front view
Quis
L-AP4
LY354740
ACPT-I
APDC
DCG-IV
b) side view
Quis
L-AP4
LY354740
ACPT-I
APDC
DCG-IV

carbonyl group (green), in the central part the LY374740 hydrocarbon cycle (yellow), the APDC cyclic nitrogen atom (magenta), the ACPT third carboxylic group (purple) and the third oxygen atom of phosphonic groups (blue), in the lower part is located the DCG-IV third acidic function (gray).

These features may allow the design of new potent and selective mGlu receptor ligands. They may also be helpful for the refinement of the LIVBP-like model of the glutamate binding site.

5. Additional regulatory sites on mGlu receptors

As described above, the glutamate binding site has been mapped in the extracellular domain of mGlu receptors (Fig. 1). However, other molecules, like Ca^{2+} and Gd^{3+} have been shown to modulate the activity or mGlu receptors (Kubo et al., 1998; Hammerland et al., 1999) (Fig. 9). One paper propose that Ca^{2+} is sufficient to activate various mGlu receptors, an effect antagonized by the competitive antagonists, like MCPG (Kubo et al., 1998). However, other authors found that Ca^{2+} potentiates the action of glutamate on $mGlu_1$ receptors by increasing its maximal effect and decreasing its EC_{50} value (Saunders et al., 1998). One residue close to the glutamate binding site has been shown to play a critical role for this Ca^{2+} effect: Ser^{166} which is conserved in all mGlu receptors except the $mGlu_2$ receptor (Kubo et al., 1998). This residue is also not conserved in the Ca^{2+}-sensing receptor, suggesting this ion is not acting the same way in this receptor and mGlu receptors.

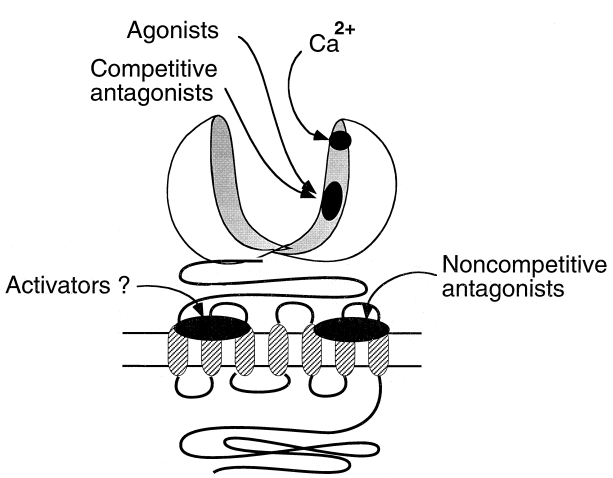


Fig. 9. Multiple regulatory sites likely exist on mGlu receptors: the agonist binding site where competitive antagonists also likely bind; a site on top of the tranmembrane region has been identified on $mGlu_1$ receptors where the non-competitive antagonist CPCCOEt binds; Ca^{2+} has also been shown to activate (or potentiate) many mGlu receptor subtypes; a site located within the transmembrane region has also been identified in the Ca^{2+}-sensing receptor where calcimimetics act. These compounds potentiate the action of Ca^{2+} on the Ca^{2+}-sensing receptor. Similar molecules may also possibly exist for mGlu receptors.

Synthetic molecules like the phenylalkylamines NPS467 and NPS568 have been shown to potentiate the action of Ca^{2+} on the Ca^{2+}-sensing receptor (Hammerland et al., 1998; Nemeth et al., 1998). Neither compound had agonist effect on its own, but both induced a left shift of the Ca^{2+} dose–response curves, indicating these compounds act as allosteric modulators of the Ca^{2+}-sensing receptor. Although these compounds have no activity on any of the mGlu receptors tested, this observation suggests that similar allosteric modulators may also exist for mGlu receptors. Moreover, Gd^{3+} has been reported to activate a truncated Ca^{2+}-sensing receptor lacking the large extracellular domain (Hammerland et al., 1999). This suggests that compounds acting on the seventh transmembrane domains region of family 3 receptors can possibly act as agonists.

Regulators of mGlu receptor activity may not only act on the large extracellular domain, but also on the seventh transmembrane domains region of these receptors. As mentioned above, CPCCOEt has been shown to be a selective $mGlu_1$ receptor non-competitive antagonist which does not interact at the glutamate binding site (Litschig et al., 1998). Chimeric and mutated $mGlu_1/mGlu_5$ receptors were constructed to identify the amino acid residues responsible for the selective action of this antagonist on $mGlu_1$ receptor (Litschig et al., 1998). It was found that two residues on top of the seventh transmembrane domain of the $mGlu_1$ receptor, Thr^{815} and Ala^{818} (which are replaced by Met and Ser in the $mGlu_5$ receptor, respectively), are indeed responsible for the selective interaction of CPCCOEt with this receptor subtype. This indicates that CPCCOEt interacts with the seventh transmembrane domains region rather than with the large extracellular domain. How does CPCCOEt antagonize the activity of the receptor? Although more work is necessary to answer that question, an hypothesis can already been proposed. When over-expressed in HEK 293 cells, a significant basal activity of the receptor can be measured. This activity, also called constitutive activity, is assumed to be the consequence of the natural equilibrium between the inactive and the active state of the receptor that occurs in the absence of agonist. We found that CPCCOEt does not inhibit this the $mGlu_1$ receptor constitutive activity, indicating that this compound does not stabilize the seventh transmembrane domains region in the inactive state (Litschig et al., 1998). Since CPCCOEt totally blocks the agonist-induced activation of the receptor, one may propose that CPCCOEt prevents the liganded extracellular domain from activating the transmembrane region.

6. Perspectives

MGlu receptors are excellent examples of receptors for which a drug design strategy can lead to the development of new specific and high affinity compounds. Their extracellular domain is similar to proteins for which a crystal structure has been determined, enabling homology model-

ing studies. Moreover, the large number of existing rigid agonists and antagonists allowed to construction of precise pharmacophore models, rarely experienced for other receptor types before. Finally, the recent development of good radioligands for group-I, and group-II mGlu receptors will enable the rapid screening of compounds acting at the glutamate binding site (Cartmell et al., 1998; Schaffhauser et al., 1998). These information associated with the screening of chemical libraries in silico, should allow the design of new specific compounds. Such a strategy is well adapted for the identification of group-II and group-III mGlu receptor agonists which, as mentioned in the introduction, have potential therapeutic applications.

Our actual knowledge on the structure–function relationship of mGlu receptors also suggests a multitude of possibilities to modulate the activity of these receptors, acting at various regulatory sites different from the glutamate binding site. Based on the actual hypothesis of the activation mechanism of these receptors, compounds that stabilize the closed state of the extracellular domain should increase the potency of agonists. Compounds interacting with the seventh transmembrane domain region may either stabilize the active state of the G-protein activating domain of the receptor, or prevent its activation by the liganded extracellular domain. Such molecules may therefore be either allosteric regulators, agonists or antagonists. Finally, compounds influencing the dimerization process of the family 3 receptors may also have interesting regulatory properties.

Although $mGlu_{1a}$ and $mGlu_5$ receptors have been shown to display a high constitutive activity, no inverse agonists (antagonists able to inhibit the constitutive activity of a receptor) for these receptors have been reported (Prézeau et al., 1996). This is surprising since in the case of the family 1 receptors, many competitive antagonists act as inverse agonists. The identification of such mGlu receptors inverse agonists will be useful for multiple reasons. Such molecules will help identifying the possible role, if any, of this constitutive activity. Moreover, such molecules may have specific effects that pure antagonists may not have, as observed for other receptors (Barker et al., 1994).

The screening for such new active molecules will need efficient high through put screening tests based on the coupling of these receptors to intracellular cascades. To that aim, the identification of mutated G-proteins which can be used to switch the natural transduction cascades of these receptors from the inhibition of adenylyl cyclase to the stimulation of phospholipase C or the activation of adenylyl cyclase will be very useful.

7. Conclusion

Taken together, it is a no risk beat to affirm that a multitude of very efficient compounds regulating the activity of mGlu receptors will be discovered within the next few years. These will allow a careful identification of the physiological roles of these receptors, with the hope to identify new possible therapeutic applications. Already, there is a great hope for new anxiolytic, analgesic and antiepileptic drugs, as well as new molecules that will suppress the drug withdrawal effects, or ameliorate the life of schizophrenic patients.

Acknowledgements

We thank Drs. L. Prézeau, J. Blahos and Joël Bockaert (CNRS, Montpellier, France) for constant support and critical reading of the manuscript. The authors gratefully acknowledge Dr. R. Kuhn (Novartis Pharma, Basel, Switzerland), for letting us know of the recent development of the new $mGlu_5$ receptor antagonists. The work of J.P. Pin and F. Acher was supported by grants from the Centre National de la Recherche Scientifique (CNRS), the European Community (Biomed2 (BMH4-CT96-0228) and Biotech2 (BIO4-CT96-0049) programs), the 'action incitative' 'Physique et Chimie du Vivant' from the French government (PCV97-115), Bayer company (France and Germany), the association Retina France and the Fondation pour la Recherche Médicale.

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