REVIEW ARTICLE

Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors

Emmanuel HERMANS*[1] and R. A. John CHALLISS†[2]

*Laboratoire de Pharmacologie, Université Catholique de Louvain (54.10), B-1200 Brussels, Belgium, and †Department of Cell Physiology and Pharmacology, University of Leicester, Maurice Shock Medical Sciences Building, University Road, Leicester LE1 9HN, U.K.

In 1991 a new type of G-protein-coupled receptor (GPCR) was cloned, the type 1a metabotropic glutamate (mGlu) receptor, which, despite possessing the defining seven-transmembrane topology of the GPCR superfamily, bore little resemblance to the growing number of other cloned GPCRs. Subsequent studies have shown that there are eight mammalian mGlu receptors that, together with the calcium-sensing receptor, the $GABA_B$ receptor (where GABA is γ-aminobutyric acid) and a subset of pheromone, olfactory and taste receptors, make up GPCR family C. Currently available data suggest that family C GPCRs share a number of structural, biochemical and regulatory characteristics, which differ markedly from those of the other GPCR families, most notably the rhodopsin/family A GPCRs that have been most widely studied to date. This review will focus on the group I mGlu receptors (mGlu1 and mGlu5). This subgroup of receptors is widely and differentially expressed in neuronal and glial cells within the brain, and receptor activation has been implicated in the control of an array of key signalling events, including roles in the adaptative changes needed for long-term depression or potentiation of neuronal synaptic connectivity. In addition to playing critical physiological roles within the brain, the mGlu receptors are also currently the focus of considerable attention because of their potential as drug targets for the treatment of a variety of neurological and psychiatric disorders.

Key words: Homer proteins, protein kinase C, receptor desensitization, receptor phosphorylation, signal transduction.

INTRODUCTION

Glutamate is now recognized as being the major excitatory neurotransmitter in the mammalian central nervous system, and is intimately involved in a plethora of neuronal and glial processes. In addition to its well documented involvement in learning and memory acquisition processes, glutamate is also known to have the potential to act as a potent endogenous neurotoxic agent that is proposed to play a critical role in the development or progression of diverse neurological disorders. Until the mid-1980s it was believed that glutamate exerted its neurotransmitter actions exclusively via a number of ionotropic glutamate (iGlu) receptor/channels, namely the NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid) and kainate receptors (for a review, see [1]). However, a series of studies in the mid-1980s demonstrated that glutamate could also stimulate phosphoinositide turnover with characteristics consistent with mediation by a G-protein-coupled receptor (GPCR) [2–4]. The search for the molecular identity of such a GPCR for glutamate culminated in two independent reports of the cloning of the receptor now classified as the type 1a metabotropic glutamate receptor (mGlu1a) [5,6]. These groups confirmed that the mGlu1a receptor could couple to phospholipase C (PLC) and other effectors by a G-protein-mediated mechanism. Furthermore, hydropathy mapping of the deduced primary structure provided evidence for a characteristic seven-transmembrane domain topology for the receptor. However, mGlu1a possessed little or no discernible identity with any other GPCR that had been cloned at that time.

Over the period 1991–1995 the cloning of seven additional mammalian mGlu receptors was reported, which shared 35–60% identity with mGlu1a, and which could be divided into three subgroups based on sequence similarities, preferred signal transduction pathways and pharmacological criteria (Table 1) [7]. Molecular cloning of these receptors has also highlighted the existence of multiple isoforms resulting from the alternative splicing of the mGlu receptor genes. These mGlu receptor splice variants differ essentially in the nature and size of their C-terminus (see Figure 1).

Alongside the molecular studies of mGlu receptors, there has been an enormous increase in research into the roles of this receptor family with respect to the regulation of key central nervous system functions. Although we perhaps have only a glimpse of the true importance of these GPCRs in the brain, a number of important roles can currently be delineated. For example, the generation of mGlu1(−/−) [8–10] and

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid; BAY36-7620, (3aS,6aS)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydrocyclopental[c]-furan-1-one; BHK, baby hamster kidney; $[Ca^{2+}]_e$, extracellular Ca^{2+} concentration; CaR, Ca^{2+}-sensing receptor; CHO, Chinese hamster ovary; CPCCOEt, 7-(hydroxylimino)cyclopropa-[b]chromen-1a-carboxylate; e2 loop (etc.), second extracellular loop (etc.); ERK, extracellular-signal-regulated protein kinase; EVH1, *Drosophila* Enabled, mammalian VASP, Wiscott–Aldridge syndrome protein homology domain; GABA, γ-aminobutyric acid; GPCR, G-protein-coupled receptor; GRK, G-protein-coupled receptor kinase; HEK, human embryonic kidney; i3 loop (etc.), third intracellular loop (etc.); iGlu receptor, ionotropic glutamate receptor; LIVBP, leucine/isoleucine/valine binding protein; MAPK, mitogen-activated protein kinase; mGlu receptor, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)pyridine; NMDA, *N*-methyl-D-aspartate; PDZ domain, postsynaptic density-95, Discs-large, Zona occludens-1 domain; PKC, protein kinase C; PLC, phospholipase C; PSD, postsynaptic density; PTx, pertussis toxin; RGS, regulator of G-protein signalling; sBim receptor, salmon bifunctional receptor; TM7 domain (etc.), transmembrane domain 7 (etc.); VOCC, voltage-operated Ca^{2+} channel.

[1] To whom correspondence should be addressed (e-mail emmanuel.hermans@farl.ucl.ac.be).
[2] e-mail jc36@le.ac.uk

Table 1 Structural, biochemical and pharmacological classification of mGlu receptors

Abbreviations: (*S*)-DHPG, (*S*)-3,5-dihydroxyphenylglycine; CHPG, (*R*,*S*)-2-chloro-5-hydroxyphenylglycine; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; (2*R*,4*R*)-APDC, (2*R*,4*R*)-aminopyrrolidine-2,4-dicarboxylate; L-AP4, L-2-amino-4-phosphonobutyrate; L-SOP, L-serine-*O*-phosphate; AIDA, (*R*,*S*)-1-aminoindan-1,5-dicarboxylic acid; LY367385, (*S*)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid; MCCG, 2*S*,1′*S*2*S*′-2-methyl-2-(2′-carboxycylclopropyl)glycine; LY341495, (2*S*)-2-amino-2-(1*S*,2*S*-2-carboxycyclopropan-1-yl)-3-(xanth-9-yl)propanoic acid; EGLU, (2*S*)-α-ethylglutamic acid; MeSOP, (*R*,*S*)-α-methylserine-*O*-phosphate; MAP4, (*S*)-2-amino-2-methyl-4-phosphonobutanoate; CPPG, (*R*,*S*)-α-cyclopropyl-4-phosphonophenylglycine.

Parameter	Group I	Group II	Group III
Receptor subtypes and splice variants identified in human (h) and rat (r); the total number of amino acids in each variant is indicated in parentheses	mGlu1a (h1194) [223], (r1199) [5,6]	mGlu2 (h872) [229], (r872) [224]	mGlu4a (h912) [231], (r912) [224]
	mGlu1b (h906) [33], (r906) [224] mGlu1c (h897) [111] mGlu1d (h908) [105,225], (r912) [105]	mGlu3 (h877) [230], (r879) [224]	mGlu4b (r683) [232] mGlu6 (h877) [233], (r877) [234]
	mGlu5a (h1180) [226,227], (r1171) [106] mGlu5b (h1212) [226,227], (r1203) [228]		mGlu7a (h915) [231,235], (r915) [236,237] mGlu7b (h922) [238], (r922) [239]
			mGlu8a (h908) [231], (r908) [240] mGlu8b (h908) [241], (r908) [242] mGlu8c (h501) [241]
Preferential signalling pathway	PLC	Adenylate cyclase (inhibition)	Adenylate cyclase (inhibition) Phosphodiesterase (mGlu6)
Selective agonists	L-Quisqualate (*S*)-DHPG CHPG (mGlu5)	DCG-IV (2*R*,4*R*)-APDC	L-AP4 L-SOP
Selective antagonists	AIDA LY367385 (mGlu1) CPCCOEt (mGlu1) MPEP (mGlu5)	MCCG LY341495 EGLU	MeSOP MAP4 CPPG
Synaptic localization	Postsynaptic and some presynaptic	Presynaptic	Presynaptic

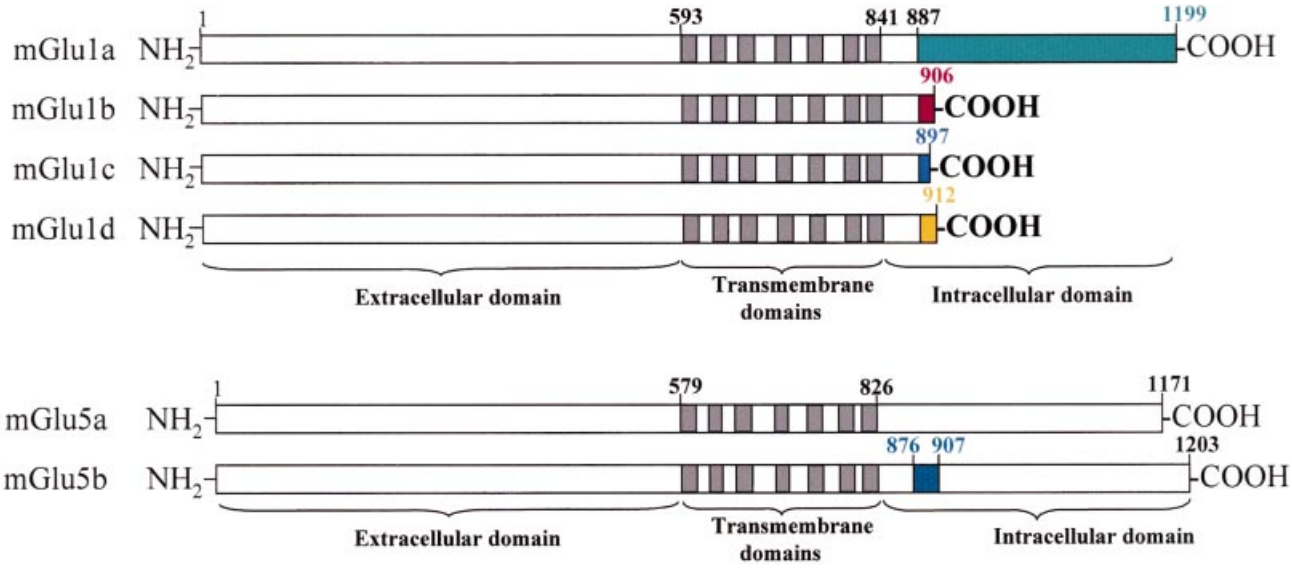

Figure 1 Schematic representation of the rat group I mGlu receptor isoforms

Grey boxes correspond to the putative seven transmembrane domains. Alternative splicing of both mGlu1 and mGlu5 mRNAs generates multiple receptors with distinct C-termini (the differences are highlighted in various colours). In the short isoforms of mGlu1, the distal 313 amino acid residues of the type 1a splice variant C-terminus are replaced by 20 (splice variant 1b), 11 (1c) or 26 (1d) residues. Compared with mGlu5a, in mGlu5b alternative splicing results in the insertion of 32 amino acid residues in the membrane-proximal region of the C-terminus. Note that the existence of additional mGlu1 isoforms has been proposed [140,217–219].

mGlu5(−/−) [11,12] knockout mice, and rescue from the knockout phenotype by transgene introduction [13], have helped to establish the specific involvement of these receptors in plastic changes in synaptic transmission leading to long-term potentiation and long-term depression. A number of excellent reviews have been published which focus upon mGlu receptor neurophysiology and pharmacology [7,14–16], and these aspects will not be considered further here. The present review will focus on

recent biochemical and molecular advances in our understanding of the structure, function and regulation of group I mGlu (mGlu1 and mGlu5) receptors.

AGONIST BINDING AND mGLU RECEPTOR ACTIVATION

Ligand recognition

In addition to the eight mammalian mGlu receptors (of which mGlu1, mGlu4, mGlu5, mGlu7 and mGlu8 exhibit splice variation), this family C (or family 3) GPCR grouping also contains $GABA_B$ receptors (where GABA is γ-aminobutyric acid; three subtypes identified to date; for a review see [17]), the extracellular calcium-sensing receptor (CaR; for a review see [18]) and a broad multigene family of olfactory, taste and pheromone receptors (more than 100 representatives; see [19,20]). Within family C GPCRs the receptors share a number of structural properties, whereas the presence of seven putative transmembrane domains is the only common feature shared with other GPCR subfamilies [21], and even within this region family C GPCRs share only 10–15 % amino acid identity with family A/B members. Family C receptors all possess a large extracellular domain (usually more than 500 residues; see Figure 2), the structure and function of which has been extensively investigated and reviewed [16,21–23].

Early studies using mutated and chimaeric receptors revealed the critical role of the mGlu receptor extracellular N-terminal domain in the recognition of mGlu receptor agonists and competitive antagonists [5,6,24–26]. Broadly similar conclusions have been reached concerning the $GABA_B$ receptor [27] and CaR [28,29]. Structural and modelling studies also revealed that the extracellular N-terminal domain of the mGlu1a receptor possesses high similarity with bacterial periplasmic amino acid binding proteins [e.g. the leucine/isoleucine/valine binding protein (LIVBP)], which are involved in amino acid uptake [24]. On the basis of molecular and structural studies, and the similarity with the bacterial amino acid binding proteins for which crystal structures were available (for a review, see [30]), a model of the glutamate recognition site was proposed in which the extracellular N-terminal domain forms two distinct facing lobes separated by a cavity where glutamate binds [22,31]. Mutagenesis studies revealed that a serine and a threonine residue (Ser^{165} and Thr^{188} in rat mGlu1), conserved in all mGlu receptor subtypes, are involved in the interaction of glutamate with the receptor [24]. More recently, the positively charged side chain of a conserved arginine residue (Arg^{78}) has been proposed to contribute to the ligand binding pocket through an interaction with an acidic group of mGlu receptor agonists [32]. Interestingly, the activities of some mGlu receptor antagonists have been reported to be dependent upon the competing agonist, indicating some variations in the molecular interaction between agonists and the extracellular domain of mGlu receptors [33,34]. A definitive demonstration that the agonist binding site lies within the extracellular region has been obtained by showing that, when expressed alone as a soluble protein [35,36], the N-terminal region of the mGlu receptor retains its ability to recognize mGlu receptor agonists.

This model of glutamate recognition by the extracellular N-terminal domain of the mGlu receptor was further supported after determination of the crystal structure of this receptor region in the presence and in the absence of glutamate [37]. From this and other supporting studies, it is proposed that the two lobes are involved in a 'Venus flytrap' mechanism of receptor activation (where glutamate binding causes the lobes to close around the ligand), and that the binding of glutamate affects the equilibrium between the closed and open states. A similar model is also proposed for other members of GPCR family C [38]. This two-lobe structure is separated from the transmembrane region of the receptor by a cysteine-rich region. Multiple roles have been proposed for this cysteine-rich region. Thus improper folding or cellular targeting of the extracellular N-terminal domain of group III mGlu (mGlu4 and mGlu8) receptors was observed when this receptor region was deleted [39], whereas its entire deletion from the CaR was shown to impair functional activation of the receptor by agonists [40]. Intriguingly, $GABA_B$ receptors lack this cysteine-rich domain [41]. According to the hypothesized model of receptor activation, involving the folding of the two lobe regions towards the seven-transmembrane domain (see below), one may also propose that the cysteine-rich region constitutes a flexible spacer allowing the displacement of the glutamate binding pocket towards the transmembrane domains.

Mechanisms of mGlu receptor activation: possible models

The location of the agonist binding site in mGlu receptors (and most probably in family C GPCRs in general) raises the question of the molecular mechanism of receptor activation. For classical family A GPCRs (e.g. rhodopsin, β_2-adrenoceptors, etc.), the binding of agonist occurs within a 'pocket' formed by the transmembrane domains [42], which induces a change in receptor conformation facilitating G-protein coupling and activation of downstream signalling pathways. For these receptors, it is also well documented that pre-coupling with G-proteins stabilizes the GPCR in a conformation possessing high affinity for the agonist (the ternary complex model). Experimentally, high-affinity agonist binding can be disrupted by addition of guanine nucleotides that bind to G-proteins and cause dissociation of the receptor–G-protein complex. It is noteworthy that, although mGlu receptor agonists do not bind directly to the transmembrane region of the receptor, they do show guanine-nucleotide-sensitive specific binding of radiolabelled agonists, including [^{3}H]glutamate [43–47]. This indicates that, despite the distance separating the glutamate binding site and the transmembrane region, these domains of the receptor may interact dynamically together upon agonist binding.

In accordance with this observation, the different mechanisms of mGlu receptor activation that have been proposed so far involve a modification of the conformation of the extracellular N-terminal domain that brings this domain closer to the transmembrane domain. By analogy with the bacterial periplasmic proteins (see above), such spatial displacement was proposed previously to enable the delivery of the bound amino acid substrate to a critical activation site of the transmembrane domain [48]. However, this model, which defines a mechanism for ligand delivery/transfer between LIVBP and a specific high-affinity transporter, is not compatible with studies conducted with chimaeric CaRs in which the receptor was modified by exchanging the extracellular N-terminal domain for this region of the mGlu1 receptor. The chimaeric $mGlu1^{1–592}/CaR^{613–1078}$ was activated efficiently by glutamate, indicating an apparent lack of specificity of the putative activation site at the level of the seven transmembrane domains [29]. It is now generally believed that the transmembrane region of the receptor is activated by the extracellular N-terminal two-lobe domain of the receptor, whose active conformation is stabilized upon agonist binding [16,49]. This model shares some characteristics with the thrombin receptor, and other protease-activated receptors (PAR1–4), for which the activator of the receptor is an N-terminal region of the receptor that becomes unmasked following protease cleavage and interacts with specific transmembrane domains to cause receptor activation [50].

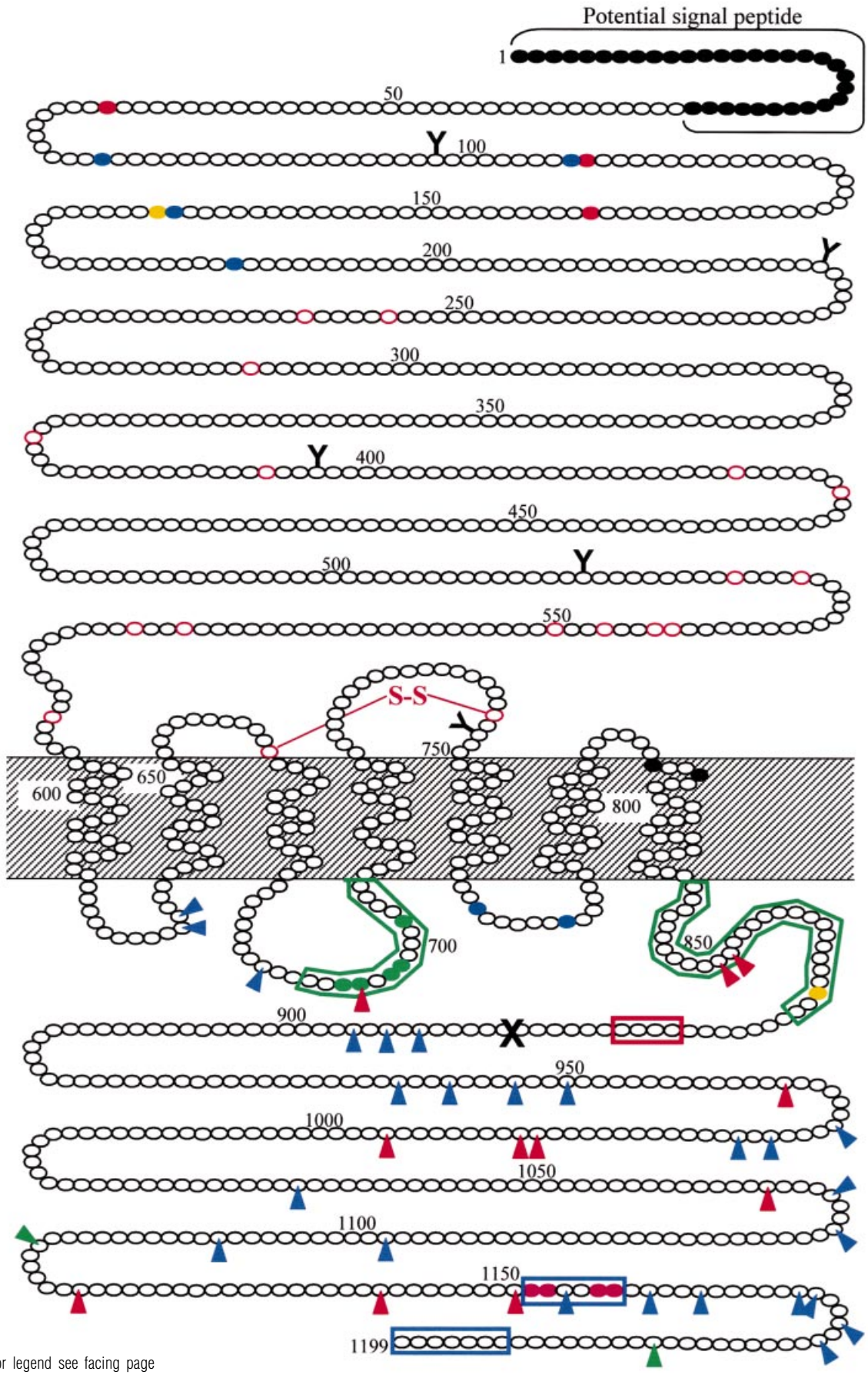

Figure 2 For legend see facing page

The model of mGlu receptor activation is further supported by the development of non-competitive group I mGlu receptor antagonists and the identification of their sites of receptor interaction. Thus a number of groups have now demonstrated that the mGlu receptor subtype-selective antagonists CPCCOEt {7-(hydroxylimino)cyclopropa-[b]chromen-1a-carboxylate} and BAY36-7620 {(3aS,6aS)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydrocyclopental[c]-furan-1-one} block mGlu1 activation non-competitively [49,51,52], while MPEP [2-methyl-6-(phenylethynyl)pyridine] has a similar non-competitive effect on mGlu5 receptors [53]. These agents can be shown not to bind at the agonist binding site [36,54,55], and the use of chimaeric mGlu1/mGlu5 and CaR/mGlu receptor constructs has provided clear evidence that the pharmacological specificity of these agents depends on critical amino acid residues present within the transmembrane segments [49,55,56]. For example, it has been shown that Thr^{815} and Ala^{818} in TM7 (transmembrane domain 7) of the mGlu1 receptor are essential for the antagonist activity of CPCCOEt, and binding at this locus presumably uncouples agonist binding from receptor activation [49]. For MPEP, amino acid residues in both TM7 and TM3 of the mGlu5 receptor appear critical [55]. Non-competitive antagonism has also been shown for agents acting at other family C GPCRs, e.g. NPS-467 [(*S*)-(−)-*N*-(3-phenylpropyl)-α-methyl-(3-methoxy)benzylamine hydrochloride] at the CaR [57].

One intriguing observation, alluded to above, is the apparent lack of specificity of the putative activation site of a receptor, located within the seven-transmembrane region, which will tolerate at least a certain degree of switching of N-terminal domains between family C receptors [27,28,56,58]. Furthermore, the proposed model for indirect mGlu receptor activation, where the agonist causes a change in the N-terminal binding lobes and the ligand-bound extracellular region then undergoes a conformational change to interact with the seven-transmembrane region, may be difficult to reconcile with the partial agonist activity shown by some mGlu receptor agonists [59,60], or antagonists that show inverse agonist activity [52] (see below). Therefore, although major progress has been made in the description of agonist and antagonist binding, further studies are still required before we can fully understand the molecular mechanisms involved in family C GPCR activation.

Constitutive mGlu receptor activation

Constitutive activity, i.e. the ability of a GPCR to facilitate GTP/GDP exchange/activation of G-proteins in the absence of agonist, has been demonstrated for a number of GPCRs in both native and recombinant model cell systems (see [61]). mGlu1a (but not mGlu1b or mGlu1c) receptors recombinantly expressed in either pig kidney epithelial (LLC-PK1) or human embryonic kidney (HEK) 293 cells exhibit constitutive activity with respect to phosphoinositide turnover, and the extent of agonist-independent activity was proportional to the level of receptor expression [62]. The possibility that the apparent constitutive activity might have arisen through the release of glutamate by the cells was excluded by the fact that mGlu receptor antagonists completely prevented agonist-stimulated mGlu1a-mediated responses, but did not suppress basal phosphoinositide turnover to levels seen in mock-transfected cells [62].

Subsequent studies have shown that the non-competitive mGlu1 receptor antagonist BAY36-7620 possesses (partial) inverse agonist activity [52], while Pagano et al. [55] have confirmed the initial observations of Prézeau and colleagues [62] with respect to the constitutive activity of mGlu5a/b receptors expressed in a HEK 293 cell background, and have shown that agonist-independent activity can be suppressed by the non-competitive mGlu5 receptor antagonist MPEP [55]. An elegant recent study has provided clear evidence that the constitutive activity of mGlu1a receptors endogenously expressed in mouse cerebellar granule cells and/or of mGlu5 receptors recombinantly expressed in this cell background is controlled through interaction with Homer proteins [63]. Thus in neuronal cells the constitutive activity of mGlu1/5 receptors is normally suppressed through interactions with cytoskeletal elements: this will be discussed further in the section on Homer proteins (see below).

Modulation of group I mGlu receptors by extracellular bivalent cations

As stated above, group I mGlu receptors belong to the same subfamily of GPCRs as the CaR, which is specifically activated by bivalent cations. An additional member of this subfamily, cloned from salmon brain, has been shown to be activated by either glutamate ($EC_{50} \sim 5\ \mu M$) or Ca^{2+} ($EC_{50} \sim 1$ mM) and has therefore been named the salmon bifunctional metabotropic (sBim) receptor [64]. The close resemblance of this receptor to the mGlu1a receptor (69% identity of amino acid sequence) raised the question of the ability of Ca^{2+} to also activate mammalian mGlu receptors. In a variety of recombinant systems where the mGlu1a receptor has been expressed, the functional response to glutamate can be shown to be affected by alterations in the extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) [64–67]. For example,

Figure 2 Structural features of the mGlu1a receptor

The N-terminal glutamate binding site possesses structural homology with bacterial proteins involved in amino acid transport [24]. Amino acid residues involved in glutamate recognition are highlighted in blue (Arg^{78}, Arg^{106}, Ser^{165} and Thr^{188}) [14,24,32]. This region of the receptor is proposed to form a two-lobed 'venus flytrap' binding pocket, and is separated from the transmembrane domain by a cysteine-rich region, whose role (if any) has yet to be clearly established. The modulation of mGlu1a activity by bivalent cations has been proposed to involve Ser^{166} [68], indicated in yellow. Disulphide bridges between cysteine residues of the extracellular domain may be responsible for homodimerization of group I mGlu receptors. Among the multiple cysteine residues present in this region of mGlu1a (open red symbols), Cys^{140} (and possibly Cys^{67} and Cys^{109}) (closed red symbols) is critical for receptor dimerization [82,220]. As for classical GPCRs, an intra-receptor disulphide bridge between the first and second extracellular loops has been proposed (Cys^{661} and Cys^{746}) [23]. N-linked glycosylation of mGlu receptors has been demonstrated [79,94,221], but specific sites of glycosylation have not been clearly assigned. Possible N-glycosylation sites are indicated by a **Y** symbol. Both the i2 and i3 loops are involved in coupling to and activation of multiple G-proteins [137,139]. Critical residues that determine the coupling of mGlu1 with G_s (Pro^{698}, Cys^{694} and Thr^{695}) and G_q (Thr^{695}, Lys^{697} and Ser^{702}) are shown in green [100]. G-protein activation requires specific residues (blue) within the i3 loop (Arg^{775} and Phe^{781}). Stretches of basic residues (green boxes) are found in the i2 loop and the membrane-proximal region of the C-terminus and contribute to PLC activation [138,139]. A cluster of four basic residues within the membrane-proximal region of the C-terminus interferes with the functional coupling of shorter mGlu1 splice variants with PLC (Arg^{877}–Lys^{880}; highlighted with a red box) [136]. Removal of these residues has also been shown to alter cell surface expression of short mGlu1 isoforms [142]. The non-competitive mGlu1 antagonists CPCCOEt and BAY36-7620 have been shown to interact with the transmembrane domains of the receptor [49]. TM7 residues Thr^{815} and Ala^{818} (closed black symbols) are specifically involved in the binding of CPCCOEt [49]. Although a consensus sequence for palmitoylation is found in the membrane-proximal region of the C-terminus (Cys^{867}; in yellow) [138], the mGlu1a receptor is not palmitoylated in transfected BHK cells [222]. The position (886–887) at which mGlu1 splicing gives rise to multiple splice variants is indicated by an **X**. Specific domains (blue boxes) of the C-terminus of group I mGlu receptor long isoforms are proposed to interact with Homer proteins [165,168]. The PPXXFR sequence (residues 1152–1157 in mGlu1a; in pink) is critical for this interaction [168]. Multiple putative phosphorylation sites (serine, blue triangles; threonine, red triangles; tyrosine, green triangles) are found in the intracellular domains (identified using the software NetPhos2.0) and are possibly involved in phosphorylation-dependent desensitization [107,187]. PKC-mediated phosphorylation of Thr^{695} has been demonstrated [201]. Further details concerning the interaction of group I mGlu receptors with diverse intracellular proteins are provided in Table 2.

reductions in $[Ca^{2+}]_e$ have been reported to decrease both the potency and the efficacy of glutamate action, whereas increases to supraphysiological $[Ca^{2+}]_e$ (from 1.3 to 4 mM) have the opposite effect [67]. Some evidence supporting a requirement for extracellualr Ca^{2+} in receptor–ligand interactions also arises from radioligand binding studies [54,60]. A recently published X-ray crystallographic structure of the N-terminus of the mGlu1 receptor has also identified a cation binding site [37], although the location of this site and its apparent very high affinity for the cation make it unlikely to be the locus for $[Ca^{2+}]_e$-dependent receptor activation.

Thus, although a number of studies have shown that the coupling of the mGlu1a receptor to PLC activation is dependent on extracellular Ca^{2+}, the activation of this receptor by extracellular Ca^{2+} *per se* remains controversial. Thus Kubo et al. [66] showed that, in *Xenopus* oocytes recombinantly expressing the mGlu1a receptor, increasing $[Ca^{2+}]_e$ resulted in a robust Ca^{2+}-activated Cl^- current, an effect that was mimicked by a number of other bivalent and tervalent cations. An increase in $[Ca^{2+}]_e$ was also found to trigger activation of the mGlu5 receptor, and to a lesser extent the mGlu3 receptor, but not the mGlu2 receptor. Further studies on chimaeric mGlu1 and mGlu2 receptors revealed a critical role for the N-terminus, and mutagenesis studies indicated that a single residue (Ser^{166}) of mGlu1, located in the immediate vicinity of the glutamate binding site, determines the Ca^{2+} sensitivity of the mGlu receptor. In transfected Chinese hamster ovary (CHO) cells, the presence of this residue in the N-terminus of mGlu receptors was also demonstrated to be involved in sustained responses to agonists observed in the presence of extracellular Ca^{2+} [68]. Further, in baby hamster kidney (BHK) cells stably expressing the recombinant rat mGlu1a receptor, $[Ca^{2+}]_e$ was also found to modulate mGlu1a receptor-mediated PLC activation positively, but, in contrast with the data obtained in *Xenopus* oocytes, extracellular Ca^{2+} *per se* did not trigger receptor activation [67].

Although the sensitivity of PLC-β to changes in the intracellular Ca^{2+} concentration is well documented [69,70], a modulatory effect of $[Ca^{2+}]_e$ on agonist-stimulated phosphoinositide turnover was not observed for another GPCR, the M_3-muscarinic receptor expressed in the same cell background, unless $[Ca^{2+}]_e$ was reduced to sub-micromolar levels in the medium [67]. Interestingly, a similar modulation of the $GABA_B$ receptor by $[Ca^{2+}]_e$ has been reported [71]. Although extracellular Ca^{2+} does not act as an agonist at the $GABA_B$ receptor, the binding affinity for some $GABA_B$ agonists was increased in the presence of extracellular Ca^{2+}, whereas no effect was observed with respect to the binding of $GABA_B$ receptor antagonists. A critical residue (Ser^{267}), present in the N-terminus of this receptor, has recently been shown to confer sensitivity to extracellular Ca^{2+} on this GPCR [72].

Despite such evidence for a modulation of mGlu1a receptor-mediated responses (and perhaps those of other family C receptors) by $[Ca^{2+}]_e$, the issue of whether this receptor possesses true 'Ca^{2+}-sensor' properties has recently been reassessed by analysing the effect of varying $[Ca^{2+}]_e$ on the immediate effector response to the addition of mGlu receptor agonists [73]. As shown previously in other models, the magnitude of the phosphoinositide response to agonist was found to be dependent on the presence of extracellular Ca^{2+} and to be substantially affected by changes in $[Ca^{2+}]_e$ in the millimolar range in CHO cells expressing mGlu1a. However, the initial increase in inositol 1,4,5-trisphosphate ($InsP_3$) and the Ca^{2+} mobilization response following mGlu receptor agonist addition (and measured within 10–15 s) were essentially similar under normal (1.3 mM Ca^{2+}) and Ca^{2+}-free conditions. In contrast, sustained $InsP_3$ accumulation was substantially reduced under Ca^{2+}-free conditions, and the steady-state increase in the intracellular Ca^{2+} concentration appeared to be directly proportional to $[Ca^{2+}]_e$. Thus varying $[Ca^{2+}]_e$ was shown to modulate the response to sustained stimulation, whereas initial activation of PLC and Ca^{2+} mobilization from intracellular stores was independent of $[Ca^{2+}]_e$. Ca^{2+} influx through Ca^{2+}-store-operated Ca^{2+} channels thus plays a critical role in the sustained response to the stimulation of the GPCR by positively modulating receptor-mediated PLC activity. It can therefore be proposed that the apparent Ca^{2+}-sensing properties of mGlu1a are most likely to be a consequence of the need for Ca^{2+} influx to sustain PLC activity and maintain elevated rates of phosphoinositide turnover; the precise physiological significance of this 'late phase' modulation is not known.

Dimerization and glycosylation of mGlu receptors, and implications for receptor activation

A number of biochemical studies using mGlu1- or mGlu5-selective antisera and Western blot analysis or immunoprecipitation have revealed the presence of high-M_r immunoreactive proteins both in tissues and in transfected cells expressing recombinant mGlu receptors [74–77]. These high-M_r immunoreactive proteins were considered to be protein aggregates, i.e. dimeric or multimeric assemblies of mGlu receptor monomers, depending on their resistance to reducing conditions. In addition, a recognized feature of the mGlu receptor subfamily (and other family C GPCRs) is the presence of multiple cysteine residues within the region of the extracellular N-terminus proximal to TM1. It has been proposed that at least some of these residues may be involved in receptor dimerization. The first demonstration of an involvement of cysteine residues in dimerization was provided for mGlu5 by showing co-immunoprecipitation of two distinct epitope-tagged forms of the mGlu5 receptor [78]. Similar conclusions were reached by Robbins et al. [79] and Okamoto et al. [36] concerning the dimerization of the mGlu1a receptor, since the N-terminus of this receptor ($mGlu1^{33–592}$; truncated at the N-terminus–TM1 junction) is secreted into the culture medium as a dimer. Multiple cysteine residues present in the extracellular N-terminal domain of CaR were also found to play a critical role in its dimerization [80,81]. Similarly, mutagenesis studies have identified the location of the cysteine residues involved in the dimerization of the mGlu1 [82] and mGlu5 [83] receptors. Despite their sequence identity, mGlu1 and mGlu5 do not appear to form heterodimers [78]. Furthermore, although a critical role for the N-terminus has been demonstrated in receptor dimerization, heterodimerization was not observed between the mGlu1a and mGlu1b receptors, which differ only in their C-termini, indicating that other regions of the receptors could be involved in the regulation of the dimerization process [79].

Other GPCRs have been demonstrated previously to exist as dimers is mammalian cells. However, dimerization of these receptors often does not appear to involve disulphide bridges between cysteine residues, but more likely results from non-covalent interactions between transmembrane-spanning domains [75,84] or intracellular domains [85]. A notable exception here is the heterodimerization of κ- and δ-opioid receptors, which appears to involve disulphide cross-linkage [86]. In the case of mGlu receptors, recent studies have shown that both covalent and non-covalent interactions contribute to dimerization [83], and this probably explains the detection of receptor dimers even under reducing conditions.

Although it is now well established that mGlu and other family C GPCRs exist as dimers at the cell surface, the role of this dimerization is still unclear. Treatment of hippocampal slices,

cerebellar granule cells or transfected fibroblasts expressing mGlu5 with reducing agents was found to decrease dramatically the functional response to agonist [87,88]. It is therefore tempting to propose a possible (patho)physiological modulation [87] of mGlu receptor activity in response to variations in the extracellular redox potential, which can be greatly altered during seizure activity or oxidative stress. However, in contrast with these studies, removal of critical cysteine residues in the extracellular domains of mGlu1 and mGlu5 did not affect the specific binding of, or the functional response to, agonist [82,83]. Discrepant results have also been obtained for the CaR, since dimerization-deficient mutants of the CaR were found either to retain binding and functional properties [89] or to prevent activation by Ca^{2+} [80], and/or to decrease agonist affinity and receptor activation kinetics [81].

Taken together, these data indicate that the dimerization of mGlu receptors may not be directly involved in the proper folding of the receptor, permitting agonist binding and functional activation. Other possible roles of dimerization still need to be explored. Dimerization has been shown to take place in the endoplasmic reticulum, and one possible explanation is that trafficking of the receptor could be affected by its oligomerization status. Also, the influence of functional activation by agonists on receptor dimerization has yet to be systematically examined. Although the other members of the family C GPCRs have also been detected as dimers, the molecular determinants and the consequences of the dimerization of $GABA_B$ receptors are radically different from those described for mGlu receptors and the CaR. It is now clearly established that heterodimerization between $GABA_B$ receptor subtypes is required for targeting of functional receptors to the cell surface [17,90,91]. In addition, this heterodimerization of distinct receptor isoforms involves specific interaction(s) between their intracellular C-termini [92]. Recently, Ciruela and colleagues [93] have presented evidence for the physical and functional interaction of mGlu1a receptors with adenosine A_1 receptors. Such interaction may result from heterodimerization, which would involve the C-terminus of the mGlu1a receptor, since shorter splice variants or truncated mGlu1a receptors failed to interact with the adenosine A_1 receptor.

Another post-translational modification that might influence mGlu receptor function is N-linked glycosylation. The mGlu1 and mGlu5 receptors possess a number of consensus sites [NX(T/S)] for glycosylation within their N-terminal domains and second extracellular (e2) loops. For example, mGlu1 has four possible sites of N-glycosylation in the N-terminal domain, and one in the e2 loop. Few studies to date have investigated the role glycosylation may play in mGlu receptor expression and function. Robbins et al. [79] reported that preventing mGlu1a glycosylation by incubation in the presence of tunicamycin did not affect the ability of the receptor to dimerize. In contrast, Mody et al. [94] used the same agent to prevent glycosylation of the mGlu1a receptor during induction of receptor expression in the CHO-lac-mGlu1a cell model [95,96], and showed that preventing this modification eliminated dimer formation and reduced the ability of the mGlu1a receptor to couple to phosphoinositide turnover, despite apparently normal trafficking to the plasma membrane.

The N-terminally truncated rat mGlu1 receptor protein comprising residues 33–522, produced using an insect expression system and studied recently by X-ray crystallography, was shown to be glycosylated, possessing two carbohydrate modifications (at Asn^{98} and Asn^{223}) [37]. These sites, together with the e2 loop consensus site, are highly conserved between the mGlu1 and mGlu5 receptors, and it will be interesting to establish whether these are the only sites that are normally glycosylated in mammalian cells endogenously expressing mGlu1, and what the consequences are for receptor dimerization, trafficking to the cell surface and receptor–G-protein coupling efficiency. Such studies should entail the systematic mutagenesis of each asparagine residue, rather than the use of tunicamycin, which can also affect receptor lipidation.

CELL SIGNALLING THROUGH mGLU RECEPTORS

G-protein activation by group I mGlu receptors

The use of a variety of mGlu-receptor-selective agonists, and recombinant systems in which a single receptor subtype is expressed, has allowed the characterization of the signalling pathways activated by the different mGlu receptors. As indicated above, group I mGlu receptors couple preferentially to the activation of phosphoinositide-specific PLC through coupling to $G_{q/11}$ proteins. However, the potential for alternative (sometimes termed 'promiscuous') coupling with multiple intracellular signalling cascades has been widely documented. Whereas functional activation of the PLC/InsP_3/Ca^{2+} pathway is always detected, additional coupling to other transduction pathways is dependent on the system in which the mGlu receptor has been characterized (Figure 3). Although alternative coupling of GPCRs is frequently believed to be the result of receptor overexpression in recombinant systems, there is now sufficient evidence to demonstrate that such multiple coupling can often be shown to be of physiological relevance and may contribute to the complexity of the cellular responses to glutamate.

The molecular cloning and heterologous expression of mGlu receptors in *Xenopus* oocytes and transfected cells revealed that some group I mGlu receptors mediate both phosphoinositide and cAMP responses simultaneously. Thus mGlu1a receptor activation increases cAMP accumulation in oocytes, and in transfected CHO [97], BHK [98,99] and HEK 293 [100,101] cells, although some workers have reported otherwise [102]. In contrast, the splice variants mGlu1b, mGlu1c and mGlu1d failed to promote cAMP accumulation [103–105]. When expressed heterologously, stimulation of mGlu5 increases cAMP generation in oocytes and LLC-PK1 cells [104], but not in CHO cells [106] or astrocytes in primary culture [107].

The question of whether mGlu1/5-receptor-mediated stimulation of adenylate cyclase reflects a direct coupling with G_s, or is secondary to PLC activation, has been addressed in a number of studies [60,97]. The absence of an mGlu5-receptor-mediated adenylate cyclase response in a cell background where the mGlu1a receptor is effective [97], and the distinct effects of pertussis toxin (PTx) on the phosphoinositide and cAMP responses [106], suggest that PLC and adenylate cyclase are regulated independently. Further evidence supporting a specific coupling of the mGlu1a receptor to G_s was provided by Francesconi and Duvoisin [100], who showed that distinct regions of the mGlu1a receptor are involved in the functional activation of different effector responses (see below).

PTx has been utilized extensively to discriminate $G_{i/o}$ protein involvement in intracellular responses triggered by GPCRs. Some early studies provided evidence that mGlu1a-mediated responses are inhibited (at least to some degree) following PTx pretreatment, e.g. in *Xenopus* oocytes [108] and in transfected mammalian cells [60,65,109], while inconsistent results have been reported in mGlu1a-receptor-expressing CHO cell lines, where phosphoinositide hydrolysis has been found to be either decreased [97] or unaffected [110] by PTx. Group I mGlu receptor subtypes and splice variants appear to be show different sensitivities to PTx [103,106,109,111], suggesting that where receptor–$G_{i/o}$

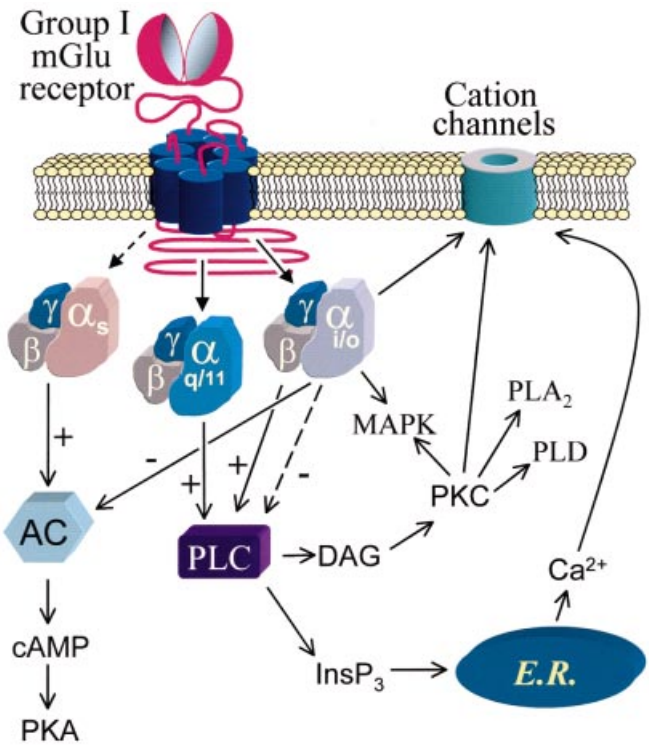

Figure 3 Multiple signalling pathways activated by the mGlu1a receptor through coupling to different G-proteins

Group I mGlu receptors couple preferentially to $G_{q/11}$-proteins, leading to activation of PLC and the consequent mobilization of intracellular Ca^{2+} and activation of PKC, via $InsP_3$ and diacylglycerol (DAG) respectively. PKC has been proposed to be involved in the activation of phospholipase D (PLD), phospholipase A_2 (PLA_2) and perhaps MAPK, as well as in the modulation of a variety of ion channels. In various models, the mGlu1a receptor has also been shown to couple to PTx-sensitive $G_{i/o}$-proteins, and such coupling may result in the inhibition of adenylate cyclase (AC), the modulation of MAPK and ion channel activities, and possibly the negative regulation of PLC activity (see main text). Group I mGlu receptor agonists have also been shown to mediate adenylate cyclase activation, indicating a possible coupling with G_s proteins, at least in some cell backgrounds (see main text). E.R., endoplasmic reticulum; PKA, cAMP-dependent protein kinase.

coupling occurs it is a genuine property of the receptor rather than a non-specific, promiscuous coupling attributable to the high level of receptor expression seen in some systems.

In contrast with the reported inhibitory effects of PTx pretreatment, we [99,112] have shown that PTx can result in a marked *enhancement* of the receptor-mediated phosphoinositide response in BHK cells stably expressing the mGlu1a receptor (see below). Additional evidence for the coupling of the mGlu1a receptor to PTx-sensitive G-proteins has been obtained through evaluation of mGlu1a-receptor-mediated guanine nucleotide exchange [113], Ca^{2+} channel activation [114,115] or activation of extracellular-signal-regulated kinase 2 (ERK2) [116]. Furthermore, the mGlu1a receptor expressed in HEK 293 cells can activate PLC efficiently via a recombinantly expressed chimaeric G_q protein in which the N-terminal sequence, crucial for receptor recognition, is substituted by the corresponding sequence from G_i [101].

In BHK cells, mGlu1a receptor activation has been shown to mediate GTP/GDP exchange at both $G_{q/11}$- and G_i-type G-proteins, as revealed by specific immunoprecipitation of $G\alpha$ subunits binding the non-hydrolysable GTP analogue guanosine 5′-[γ-[^{35}S]thio]triphosphate [99,110]. The consequence of receptor–$G_{i/o}$-protein coupling was shown to be an antagonism of $G_{q/11}$-mediated PLC activation. This phenomenon was not observed in another recombinant system (CHO cells expressing mGlu1a at a lower level) [110], suggesting that the extent and consequences of mGlu1a receptor activation of $G_{i/o}$-proteins may vary depending upon cell background and/or expression level.

A functional role for PTx-sensitive G-proteins in the cellular responses to activation of group I mGlu receptors has perhaps been most widely documented with respect to the regulation of ion conductances [7]. Receptor–ion-channel modulation can be mediated downstream of second messenger generation, or can be 'membrane delimited', implying a direct ion-channel modulation by $G\alpha$ or $G\beta\gamma$ subunits. Perhaps the best characterized example of membrane-delimited mGlu receptor modulation is the predominantly PTx-sensitive modulation by mGlu1/5 of voltage-operated Ca^{2+} channels (VOCCs) that has been demonstrated in both neurons and recombinant cell systems [114,115,117]. Thus mGlu1 and mGlu5 receptors, co-expressed recombinantly with either $\alpha_{1A}/\alpha_2\delta/\beta_{1b}$ or $\alpha_{1B}/\alpha_2\delta/\beta_{1b}$ (components of the P/Q- and N-type VOCCs respectively) in HEK 293 cells, can negatively modulate Ca^{2+} conductance with characteristics of mediation by $G_{i/o}$-protein-derived $\beta\gamma$-subunits [115]. In contrast, inhibition by the mGlu1a receptor of the M-type K^+ conductance (I_M) is PTx-insensitive [114], and although mGlu1/5 receptors transiently activate G-protein-coupled inwardly rectifying K^+ channels via a PTx-sensitive mechanism, the predominant effect is inhibitory and is mediated via a PTx-insensitive PLC/protein kinase C (PKC)-dependent indirect pathway [118].

Another family of proteins that may govern the activation of different G-protein subpopulations by mGlu receptors are the 'regulators of G-protein signalling' (RGS) proteins [119,120]. These proteins were first characterized through their ability to affect the lifetime of $G\alpha$–GTP, and hence the duration (and kinetics) of effector activation. RGS proteins appear to be widely and differentially expressed in the brain [121]. To date, relatively few studies have investigated the roles of RGS proteins with respect to group I mGlu receptor function; however, these limited data already give an insight into their potential importance [23,114,122].

In addition to the regulation of PLC/adenylate cyclase and a variety of ion channels, group I mGlu receptors have also been shown to interact, directly or indirectly, with a number of other cellular effector molecules (see also below). Thus, in addition to activation of PLC-β, mGlu1a has also been shown to stimulate arachidonate formation, presumably through activation of a phospholipase A_2 activity [97]. In these experiments in CHO-mGlu1a cells, arachidonate release was completely inhibited by PTx pretreatment, in contrast with a partial inhibition of inositol phosphate accumulation and a facilitation of cAMP accumulation [97]. A number of studies have reported an mGlu-receptor-mediated stimulation of phospholipase D activity in both neuronal and glial preparations [123–127]. Although the activation of phospholipase D by mGlu receptor agonists is at least partially PKC-dependent and has other characteristics of a group I mGlu-receptor-mediated effector response, there are significant anomalies that have led some workers to suggest that phospholipase D activation is mediated by an as yet uncharacterized mGlu receptor [128].

Finally, group I mGlu receptors have been reported to affect cellular Ca^{2+} homoeostasis via other signalling pathways. In mouse cerebellar granule cells, mGlu1 receptors have been shown to mediate a large, oscillatory increase in the L-type VOCC conductance [129]. Although receptor coupling to this response appears to involve an intermediary G-protein, it occurs in-

dependently of PLC/InsP_3 and instead involves the promotion of a close conjunction between ryanodine receptors and L-type VOCCs [129,130]. Experimental evidence supporting similar, though not identical, mechanisms involving mGlu receptor modulation of ryanodine receptor function has also been presented [131–133].

In summary, group I mGlu receptors are able to modulate the activity of multiple intracellular signalling pathways through functional coupling with a variety of G-proteins (Figure 3). This alternative coupling, which has been widely documented in recombinant systems, can also be readily observed in the central nervous system, and is likely to depend on the nature of signalling partners present in the cell or within the immediate vicinity of the receptor. These different signalling cascades may converge (either positively or antagonistically) to modulate the same biochemical event, allowing a fine tuning of the global cellular response to glutamate [115,134]. Furthermore, the involvement of these different signalling pathways is also likely to be regulated by multiple factors that contribute to the complexity of glutamate signalling through mGlu receptors. It is also possible that interactions between mGlu receptors and other signalling proteins (including iGlu receptors) may contribute to the complex shaping of the response elicited by these receptors.

Delineation of the regions of mGlu receptors involved in G-protein coupling and activation

Although mGlu receptors possess the characteristic seven-transmembrane domain structure of the GPCR superfamily and interact functionally with G-proteins, these receptors do not share any significant sequence similarity with receptors belonging to GPCR families A and B. For prototypical family A receptors (e.g. rhodopsin, β_2-adrenoceptor) the intracellular domains of the receptor involved in functional interactions with G-proteins have been clearly delineated, and although no consensus sequence for G-protein coupling has been identified so far, mutagenesis studies have indicated a critical role for the third intracellular (i3) loop and the membrane-proximal C-terminal region [135]. Structural analyses have indicated that the corresponding regions of mGlu receptors are unlikely to play a similar discriminatory role in receptor–G-protein coupling. In particular, the TM5 and TM6 domains of mGlu receptors are connected by a short (13-amino-acid) i3 loop, the sequence of which is well conserved among the group I–III mGlu receptors that couple to distinct G-protein subpopulations. In addition, many mGlu receptor subtypes show variability at their C-terminus as a result of alternative splicing. Although this alternative splicing is sometimes associated with discernible changes in signalling properties [62,136], G-protein coupling preferences are generally well conserved. This is well illustrated by mGlu1 receptor variants, which all activate $G_{q/11}$ and PLC despite dramatic differences in their C-termini.

Receptor mutagenesis studies have contributed to the identification of mGlu receptor domains involved in G-protein/PLC coupling. By constructing chimaeric receptors between different mGlu receptor subtypes known either to activate PLC or to inhibit adenylate cyclase, Pin and colleagues [137,138] elegantly demonstrated that the i2 loop and the membrane-proximal C-terminal region are responsible for PLC activation. These regions contain several basic residues that have been proposed to form amphipathic α-helices, typical of G-protein coupling domains [138,139]. Distinct roles for the i2 and i3 loops in the selectivity of G-protein coupling and activation have been demonstrated by single amino acid substitutions within these domains. Thus Francesconi and Duvoisin [100] identified distinct critical residues in the i2 loop that are involved in the interaction of the mGlu1a receptor with either G_s or G_q. Furthermore, mutation of another critical residue (Lys^{690}) in the i2 region of the receptor was shown to 'switch' coupling to members of the G_i-protein family. Complementary to these observations was the finding that the i3 loop possesses crucial residues for the activation of G_s and G_q. Thus, whereas the i3 loop is pre-eminent in governing G-protein recognition in family A GPCRs, with i1 and i2 providing a less clearly defined domain interface between receptor and G-protein, the i2 loop fulfils this crucial role in mGlu receptors, with the i1 and i3 loops playing subordinate roles [140].

Influence of C-terminal splicing on mGlu receptor signalling

Possible differences in the functional responses evoked by the splice variant forms of both mGlu1 and mGlu5 receptors have been widely investigated. As indicated above, the mGlu1 splice variants show considerable variability in the length of their C-terminus (Figure 1). A number of studies have shown that the presence of a short C-terminus (as present in mGlu1b, mGlu1c and mGlu1d) is associated with reduced agonist potency and slower agonist-stimulated second messenger responses compared with the full-length (mGlu1a) receptor [104,105,111]. Further, it has been proposed that the long C-terminal domain of the mGlu1a receptor increases receptor–PLC coupling efficiency [62,105]; however, more recent studies have indicated that a truncated mGlu1 receptor (Δ879-mGlu1), lacking the entire C-terminal domain beyond the splicing site, shows signalling properties almost identical with those of the full-length mGlu1a receptor [136]. In addition, mutagenesis studies have identified a cluster of four basic residues (RRKK; Arg-Arg-Lys-Lys) that is present in all mGlu1 splice variants (but is bisected in the Δ879-mGlu1 mutant) and which impairs the coupling of the shorter receptor isoforms to PLC. Since this sequence is also present in the mGlu1a receptor, it has been proposed that the inhibitory effect of the RRKK motif is masked by the presence of the long C-terminus. Interestingly, truncation of the mGlu1a receptor has been shown previously to 'unmask' an inhibitory effect of this receptor on adenylate cyclase activity, providing further evidence for a role for the C-terminus of mGlu1 receptors in their coupling specificity [65]. Another particular feature of the group I mGlu receptor splice variant possessing a long C-terminus is the report of 'constitutive' receptor activity that has not been observed in cells expressing the shorter isoforms [62,104,105,141].

Very recently, an alternative function of the RRKK cluster has been suggested [142]. Chan and colleagues propose that the RRKK motif is an endoplasmic reticulum retention sequence which results in low levels of an incompletely glycosylated mGlu1b receptor being expressed in HEK 293 cells. Whereas in neuronal cells the mGlu1b receptor is trafficked appropriately to the cell surface, recombinant expression in HEK 293 cells is severely compromised unless the RRKK domain is masked by the mGlu1a receptor C-terminus. These data suggest that, at least in neurons, other proteins are responsible for masking the RRKK sequence and chaperoning the receptor to its appropriate plasmalemmal location [142].

The mGlu5a and mGlu5b receptors differ only by a 32-amino-acid insertion in the C-terminus, and no shorter splice variants of mGlu5 have been identified. The mGlu5 isoforms are expressed differentially during development and have distinct distributions in the adult central nervous system [143]. Although no differences with respect to mGlu5a/mGlu5b signalling mechanisms or pharmacology have been reported [104,144], these mGlu receptor isoforms exhibit different, overlapping localizations within neurons and can differentially influence neuronal (neurite) architecture [144].

Table 2 Intracellular proteins interacting with group I mGlu receptors

Interacting protein	Interacting sites	Roles and/or consequences
G-proteins	i2 and i3 loops	Discriminative coupling with intracellular and membrane effectors
Homers	Proline-rich domain of the intracellular C-terminus of long isoforms	Adaptor proteins controlling molecular interactions with signalling proteins, e.g. Ins(1,4,5)P_3 receptors [168], ryanodine receptors [130]; may also be involved in receptor trafficking [170–174], in interactions with the PSD proteins [176] and other cytoskeletal elements [243], and in the modulation of the functional coupling of the receptor with ions channels [178]
Calmodulin	Two binding sites in the membrane-proximal region of mGlu5 receptor (separated by the mGlu5b-specific alternative exon)	Modulation of mGlu receptor signalling by interfering with its phosphorylation [102]
Tubulin	Within the last 86 amino acids of the C-terminus of mGlu1a receptor	mGlu receptor clustering [244,245] and anchoring to the plasma membrane [246]
Src-family protein tyrosine kinase	Proline-rich domain of the intracellular C-terminus of long isoforms	G-protein-independent intracellular signalling [147]
Arrestins	Not determined	Control of homologous desensitization of the receptor and endocytosis following phosphorylation [155]

G-protein-independent signalling by group I mGlu receptors

Although the vast majority of downstream signalling events initiated by ligand binding to GPCRs can be shown to be dependent on receptor-mediated facilitation of GTP/GDP exchange at heterotrimeric G-proteins, it is becoming increasingly evident that GPCRs can also interact with other cellular proteins, in some cases leading to G-protein-independent signalling by GPCRs [145,146]. A possible G-protein-independent modulation of a membrane ion conductance by the mGlu1 receptor in hippocampal pyramidal neurons has been reported recently [147]. An analysis of the transduction mechanism linking mGlu1 receptor activation to modulation of the excitatory postsynaptic current provided evidence for the involvement of an Src-family non-receptor tyrosine kinase. Interestingly, another GPCR, the β_3-adrenoceptor, has been shown to bind c-Src directly via proline-rich (PXXP) motifs in the i3 loop and C-terminus of the receptor [148], leading to activation of ERK. The mGlu1a, mGlu5a and mGlu5b receptors all possess proline-rich regions (including multiple PXXP motifs) within their long C-terminal domains, suggesting that c-Src may interact with this region of the receptor (Table 2).

One of the first examples of G-protein-independent signalling by GPCRs was the observation that receptor phosphorylation by G-protein-coupled receptor kinases (GRKs) and subsequent binding of β-arrestin does not simply uncouple GPCRs from further G-protein activation, but for some GPCRs also initiates an alternative signalling pathway leading to activation of mitogen-activated protein kinase (MAPK) [149]. The linkage of GPCRs to MAPK activation often involves c-Src [150] and requires receptor endocytosis [151]. Group I mGlu receptors have been shown to activate ERK in a number of preparations [116,152–154], to interact with arrestins [155] and to undergo agonist-dependent endocytosis [156]. Although other $G_{(i)}$-protein-dependent cascades have already been implicated in coupling group I mGlu receptors to MAPK activation [116], it will also be of considerable interest to determine whether these family C GPCRs utilize G-protein-independent mechanisms to bring about MAPK activation in a neuronal cell background.

Interaction of group I mGlu receptors with Homer proteins

A diverse family of proteins has been identified the members of which possess one or more PDZ [postsynaptic density (PSD)-95, Discs-large, Zona occludens-1] domains. PDZ domains allow protein–protein interactions to occur between PDZ-containing proteins and an array of proteins that contain short amino acid sequences [e.g. E(S/T)DV] often present at the extreme C-terminus of the interacting protein. In neurons, proteins possessing PDZ domains are often localized to the PSD, where they are believed to play a critical role in the organization and assembly of supramolecular signalling complexes, e.g. by forming direct or indirect physical links between cell surface receptors, ion channels and key signal transduction elements (for reviews, see [157,158]). Direct interactions of glutamate receptors with PDZ-domain-containing proteins were initially demonstrated for the NR2 subunits of NMDA receptor complexes through interaction with the PDZ protein PSD-95 [159]. This interaction was shown to contribute not only to the synaptic localization and clustering of NMDA receptors, but also to physical interactions with other proteins [160,161]. The AMPA and kainate subclasses of iGlu receptors have also been shown to interact with PDZ-domain-containing proteins [162,163].

Like the iGlu receptors, group I mGlu receptors also display a characteristic localization at the postsynaptic membrane of glutamatergic synapses. Thus group I mGlu receptors have been shown to occur perisynaptically, forming an annulus around a central core of iGlu receptors [164]. A clue as to how mGlu1 and mGlu5 receptors might be tethered at postsynaptic specializations came with the identification of a new family of proteins named Homer (or Vesl) [165,166]. The identification of the first member of the family (Homer 1a) was followed rapidly by the molecular cloning of three different genes (*homer1–homer3*), which can give

rise to many different Homer protein isoforms (see [130,167]). Homer proteins possess a PDZ-like domain and an EVH1 (Ena/VASP homology)-like domain, with the latter having been shown to interact with group I mGlu receptors. Thus Homer proteins bind to a distal, proline-rich region of the long C-terminus of the mGlu1/5 receptor, and a Homer binding consensus sequence (PPXXFR) has been predicted from site-directed mutagenesis studies [168]. The PPXXFR sequence is found in mGlu5 (isoforms a and b) and in mGlu1a, but is absent from the short splice variant mGlu1 isoforms. Putative Homer protein binding sites have also been identified in other proteins, including some other GPCRs, the InsP_3 and ryanodine receptors and phosphoinositide 3-kinase [130].

Although Homer proteins contain a single EVH1 domain at their N-terminus that allows a single contact with a binding partner, the majority of Homer isoforms also contain a conserved C-terminal coiled-coil domain that permits self-association [167,169,170]. Such association is likely to be crucial, as it allows Homer protein homo- (and hetero-) dimerization, and hence the cross-linking of mGlu receptors with other intracellular proteins (Table 2). Thus dimerized Homer proteins have been shown to couple group I mGlu receptors to InsP_3 receptors and thereby to co-ordinate the machinery of InsP_3 generation with the release of Ca^{2+} from the endoplasmic reticulum [168]. The Homer binding sequence (PPKKFRD) is conserved in all InsP_3 receptor isoforms (InsP_3R1–InsP_3R3) and is found close to the cytoplasmic N-terminal (amino acids 48–55 in InsP_3R1) of the receptor [168].

The first Homer isoform to be identified (Homer 1a) lacks a coiled-coil domain and therefore cannot dimerize. In contrast with other Homer isoforms, which appear to be constitutively expressed [169], Homer 1a is the product of an immediate-early gene whose expression is thought to be normally very low, but is readily induced, for example by intense neuronal activity (e.g. following seizure activity). One possible role for Homer 1a is as a physiological 'dominant-negative', with Homer 1a competing with other dimeric Homer isoforms to disrupt the coupling of mGlu receptors with intracellular proteins [169,170] and, perhaps as a consequence, altering intracellular signalling [130]. A recent study by Ango and colleagues [63] has clearly demonstrated that up-regulation of Homer 1a in mouse cerebellar granule cells (caused by pharmacological co-stimulation of NMDA and kainate receptors) leads to an increase in mGlu1a–Homer 1a association, a decrease in mGlu1a–Homer 3 association, and an unmasking of the agonist-independent, constitutive activity of the mGlu1a receptor over a subsequent 12 h period [63]. These data provide evidence that different Homer isoforms compete for binding to mGlu1/5 receptors, but also, dependent on their potential to dimerize, determine the constitutive activity of the receptor.

Although the precise roles of the different Homer protein isoforms remain largely undetermined, a number of important functions of Homer proteins in neurons have been suggested, and supporting experimental evidence is starting to accumulate. Thus, in addition to an anchoring role of Homers, these proteins may also act as 'chaperones', affecting the level of mGlu receptor cell surface expression [171,172], and also the subcellular localization (as well as the clustering) of mGlu receptors. The emerging picture regarding the role of Homers in targeting receptors to the cell surface is at present confusing. Thus cell surface expression of mGlu1a (and functional responses to agonist stimulation) has been reported to be enhanced after recombinant co-expression with Homer 1a [171] or Homer 1c [173] in HEK 293 cells, whereas co-expression of Homer 1b (but not Homer 1a) with the mGlu5 receptor in HeLa cells caused retention of the receptor in the endoplasmic reticulum, and a consequent marked decrease its cell surface expression [172]. Homer proteins may also affect the rate at which mGlu receptors are internalized [173].

The use of cultured neurons has allowed the proposed 'trafficking' role of Homer proteins to be addressed. The picture that is emerging strongly suggests that Homer proteins can profoundly influence the cellular localization of mGlu1/5 receptors. Thus co-expression of the mGlu5 receptor with Homer 1b or 1c in cerebellar granule neurons [174], or of Homer 1c and the mGlu1a receptor in primary cortical neurons [173], results in a predominant dendritic localization of the receptor, whereas Homer 1a co-expression favours both dendritic and axonal targeting of the receptor [174]. Further complex interactions are likely to anchor mGlu-receptor–Homer complexes at particular loci within the neuron. Thus PDZ-domain-containing proteins have been identified (e.g. Shank [175,176]) that form the 'jigsaw pieces' linking mGlu and iGlu receptors at glutamatergic synapses [177].

In neurons, Homer proteins capable of oligomerizing (the so-called 'long forms') have been shown to be involved in the clustering of mGlu1 and mGlu5 receptors, whereas Homer 1a either antagonizes or reverses the formation of such assemblies [170,174,178]. Thus long-form Homer proteins (1b, 1c and 2b) have been shown recently to modulate the cellular responses to mGlu5 receptor activation by affecting the ability of this receptor to couple to N-type Ca^{2+} and M-type K^+ channels, while Homer 1a exerted an opposite effect [178]. Such a dynamic interplay between Homer isoforms suggests that these proteins could be involved in synaptic development [166,179] or the remodelling of synapses that contributes to the long-term changes in synaptic efficacy seen at some glutamatergic synapses [166,180].

GROUP I mGLU RECEPTOR PHOSPHORYLATION AND REGULATION

A widely observed feature of the GPCR superfamily is the attenuation of the receptor-stimulated signal output upon sustained or recurrent agonist stimulation. This process, termed desensitization, has been studied in detail for a number of GPCRs, most notably rhodopsin and the β_2-adrenoceptor, and involves an initial phosphorylation of the GPCR, leading to an uncoupling of the GPCR from G-proteins. This rapid initial event (occurring on a time scale of seconds to minutes) is normally followed by receptor internalization (sequestration) and the eventual resensitization (dephosphorylation/recycling to the plasma membrane) or down-regulation (degradation) of the GPCR, which occur on a time scale of minutes to hours (for reviews, see [181,182]). Desensitization can occur via two distinct mechanisms. Second-messenger-regulated protein kinases (e.g. cAMP-dependent protein kinase and PKC) can phosphorylate GPCRs possessing the appropriate consensus sequences for serine/threonine phosphorylation within their intracellular loops and C-terminus, and this can occur irrespective of the activation status of the GPCR. In contrast, GRKs phosphorylate only activated GPCRs, and this latter process is often referred to as homologous desensitization. GRK-mediated receptor phosphorylation has been shown to increase the affinity of the receptor for another family of proteins, the arrestins, which not only sterically occlude receptor–G-protein coupling, but in many cases also act as adaptors for receptor sequestration (see [181,182]).

Involvement of PKC in desensitization of group I mGlu receptors

Early studies on mGlu receptors reported that, like other GPCRs, the group I mGlu receptors undergo desensitization upon continuous or repeated agonist exposure. Thus attenuation of mGlu-

receptor-coupled phosphoinositide hydrolysis could be demonstrated in cerebellar neurons [183,184] and hippocampal slice preparations [185]. These and other studies provided evidence for the involvement of PKC in mGlu1/5 receptor desensitization. Thus acute exposure to PKC-stimulatory phorbol esters was shown to result in a marked attenuation of agonist-stimulated phosphoinositide responses in neuronal [186] and glial [107] preparations, and in cell lines recombinantly expressing either mGlu1 or mGlu5 receptors [60,97].

The ability of PKC to phosphorylate the group I mGlu receptors has been demonstrated directly by Alaluf et al. [76]. Using a BHK cell line stably expressing recombinant mGlu1a receptors, it was shown that a rapid increase in receptor phosphorylation occurred following agonist addition, and this could be abolished in the presence of the PKC inhibitor Ro 31-8220. More recently, mGlu5 receptor phosphorylation by PKC has been implicated as a major mechanism of rapid desensitization [187]. A similar rapid desensitization of the mGlu5a and mGlu5b receptors could be demonstrated using a *Xenopus* oocyte expression system. In agreement with the previous study on the mGlu1a receptor, mGlu5a receptor desensitization was attenuated by application of PKC inhibitors and exacerbated by phorbol ester addition. Gereau and Heinemann [187] went on to explore the effects of 24 mGlu5a receptor point mutations designed systematically to remove each of the PKC consensus phosphorylation sites in the i1 and i2 loops and the C-terminal domain of the receptor. These studies identified five putative phosphorylation sites (Thr^{606}, Ser^{613}, Thr^{665}, Ser^{881} and Ser^{890}) the mutation of which partially attenuated agonist-induced receptor desensitization. Furthermore, creation of an mGlu5a receptor mutant in which all five sites were replaced by neutral (Gly/Ala/Val) amino acid substitutions produced a non-desensitizing receptor [187].

In addition to a possible role in classical desensitization of the mGlu receptor, PKC has also been proposed to mediate a rapid 'on/off switch' of the mGlu5 receptor, which might be responsible for the oscillatory Ca^{2+} signals often generated by activation of this mGlu receptor subtype. In transfected cells [188], primary astrocytes [189] and neocortical slices [190], mGlu5 receptor activation has been shown to evoke oscillatory Ca^{2+} responses. In rat hippocampal astrocytes, these intracellular Ca^{2+} oscillations have been shown to correlate with the oscillatory translocation and activation of PKCγ, a conventional diacylglycerol- and Ca^{2+}-regulated PKC isoenzyme [191], and it is proposed that these characteristic responses result from successive cycles of PKC-mediated phosphorylation/phosphatase-mediated dephosphorylation of a threonine residue (Thr^{840}) within the C-terminal domain of the mGlu5 receptor [188,189]. The mGlu1a receptor subtype does not possess a threonine residue at the corresponding position in its sequence (Asp^{854}) and, when expressed in a similar cell background to the mGlu5a receptor, mediates peak-and-plateau changes in the intracellular Ca^{2+} concentration [192]. Introduction of a threonine residue at the appropriate position in the mGlu1a receptor sequence (^{D854T}mGlu1a) converted the peak-and-plateau response into an oscillatory pattern of Ca^{2+} responses. However, a very recent study has called into question the obligatory involvement of PKC-dependent receptor phosphorylation in the repetitive sensitization and desensitization of the mGlu5 receptor [193]. Using HEK 293 cells transiently transfected with rat mGlu1a or mGlu5a cDNA, these workers have shown slow agonist-stimulated Ca^{2+} oscillatory responses (0.5 and 1.5 baseline oscillations/min for mGlu1a and mGlu5a respectively) mediated by both group I mGlu receptors. The frequency of Ca^{2+} oscillations was shown to be affected by mutation of Thr^{840} in mGlu5 and Asp^{854} in mGlu1; thus the ^{D854T}mGlu1a mutant causes a Ca^{2+} oscillatory frequency similar to that observed for the wild-type mGlu5 receptor, while mutational and pharmacological manipulations to prevent PKC-mediated receptor phosphorylation did not alter oscillatory behaviour. Thus these workers have proposed that the Ca^{2+} signatures generated by mGlu1/5 receptors are attributable to intrinsic receptor subtype/G-protein coupling properties [193].

At present it is unclear how the Ca^{2+}-oscillatory response to mGlu5 receptor activation affects (or is affected by) the PKC-mediated desensitization brought about by phosphorylation of the serine/threonine residues highlighted by Gereau and Heinemann [187]. It is possible that the two mechanisms work independently, with the progressive phosphorylation of Thr^{606}, Ser^{613}, Thr^{665}, Ser^{881} and/or Ser^{890} attenuating the Ca^{2+}-oscillatory signal, which may or may not reflect rapid phosphorylation/dephosphorylation of Thr^{840} in the mGlu5 receptor (see above). From a physiological perspective it is interesting to note that the mGlu1 and mGlu5 receptors are expressed differentially within the brain and are likely to fulfil distinct roles. Despite their common coupling to the PLC/InsP_3/Ca^{2+} signalling pathway, the distinct Ca^{2+} signalling patterns that each receptor can generate, through the different effects of PKC-mediated phosphorylation on the two subtypes, may make it possible for the mGlu1 and mGlu5 receptors to differentially regulate key aspects of neuronal activity [192]. For example, with respect to Ca^{2+} effects on transcriptional control, the frequency as well as the amplitude of changes in Ca^{2+} concentration has been shown to be important in determining the pattern of transcription factor activation and gene expression [194–197].

Other findings may also further complicate the picture with respect to the regulation of group I mGlu receptor function by PKC. Thus Minakami et al. [102] have demonstrated that calmodulin can bind to the mGlu5 receptor, and that the calmodulin–mGlu5 interaction is affected by (and can in turn influence) phosphorylation of the receptor by PKC (Table 2). The calmodulin interaction is Ca^{2+}-dependent and appears to involve two distinct sites within the intracellular C-terminus of the receptor. Both calmodulin binding sites contain serine/threonine residues within consensus sequences for phosphorylation by PKC (although not the Ser^{881} and Ser^{890} sites implicated by Gereau and Heinemann [187] in PKC-mediated receptor desensitization). Furthermore, PKC-dependent receptor phosphorylation can be inhibited by calmodulin and, conversely, phosphorylation of the receptor appears to prevent its interaction with calmodulin. The role of calmodulin binding to the mGlu5 receptor (and whether similar interactions occur with the mGlu1 receptor) requires further clarification. However, it is of interest to note that it has recently been shown that, by binding to intracellular regions of the μ-opioid and D_2 dopamine receptors, calmodulin can interfere specifically with G-protein activation, providing supporting evidence for a regulatory role for this interaction at the level of GPCR signalling [198,199].

Considerable evidence has accumulated showing that postsynaptic group I mGlu receptors are often co-expressed with iGlu receptors (see above), and recent evidence suggests that iGlu receptor activity may influence mGlu5 receptor signalling. By co-expression of the mGlu5 receptor with NR1/NR2B NMDA receptor subunits in *Xenopus* oocytes, it has been demonstrated that NMDA receptor activation reduces mGlu5 receptor desensitization [200]. This study also utilized hippocampal slice and cultured neuronal preparations, and included a direct assessment of mGlu5 receptor phosphorylation in cortical neurons. The authors demonstrated that activation of the NMDA receptor causes mGlu5 receptor dephosphorylation, and pro-

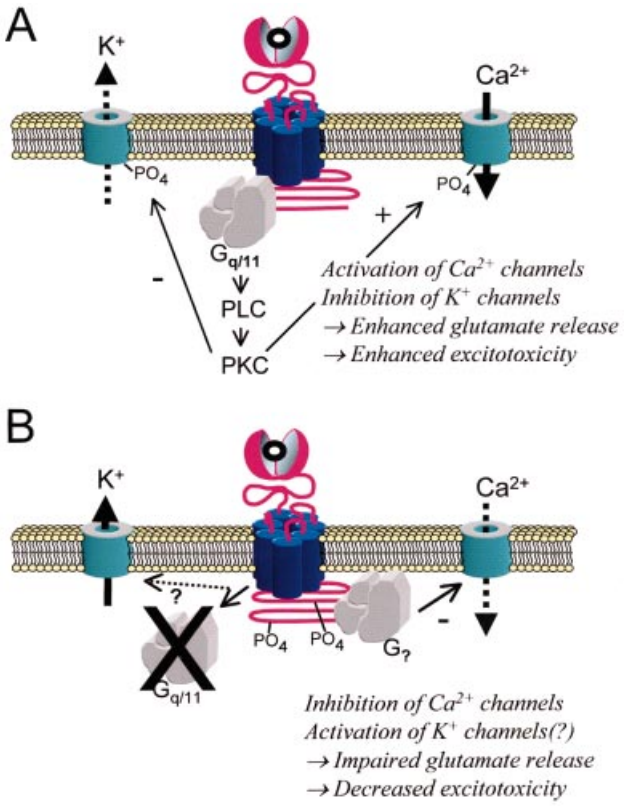

Figure 4 Possible mechanism underlying the 'switch' from facilitation to inhibition of glutamate release by presynaptic group I mGlu receptors

(**A**) Stimulation of the 'naïve' receptor triggers activation of PKC, which in turn mediates a phosphorylation-dependent inhibition of K^+ channels, and activation of VOCCs. This results in a facilitation of glutamate release, which under appropriate (ischaemic) conditions will enhance excitotoxicity. (**B**) Prolonged or repeated exposure to mGlu receptor agonist uncouples the group I mGlu receptor from the PLC/PKC signalling pathway, probably through receptor phosphorylation interfering with the receptor–$G_{q/11}$-protein interaction. However, receptor desensitization is selective, as the receptor either continues to signal via, or switches to, other G-proteins that mediate Ca^{2+} channel inhibition. This can result in an inhibition of glutamate release, and therefore has the potential to decrease excitotoxicity during cerebrovascular ischaemia (see main text for details and references).

posed that this was mediated by the Ca^{2+}-activated protein phosphatase calcineurin.

As indicated above, group I mGlu receptors appear to be capable of coupling to multiple G-protein subfamilies, in both recombinant and native systems. Aramori and Nakanishi [97] demonstrated the activation of a number of signalling pathways by mGlu1a receptors, suggestive of coupling to $G_{q/11}$-, G_s- and G_i-proteins in CHO cells. An interesting observation made by these workers was that, while phorbol ester treatment decreased (but did not abolish) the PLC-mediated response, little effect was seen with respect to agonist-stimulated cAMP (through adenylate cyclase) or arachidonate (through phospholipase A_2) accumulation, suggesting that PKC activation might differentially affect the ability of the mGlu1a receptor to couple to different G-proteins. More recent studies have shown this indeed to be the case. Thus Francesconi and Duvoisin [201] have demonstrated that, while phosphorylation of the mGlu1a receptor by PKC desensitizes the receptor with respect to phosphoinositide hydrolysis, receptor-stimulated cAMP accumulation is unaffected. Previous work by these authors had highlighted key residues within the i2 and i3 loops of mGlu1a in the selective coupling and activation of G-proteins [100]. A point mutation in mGlu1a (T695A) created a receptor that was resistant to desensitization of the mGlu1a/$G_{q/11}$/PLC pathway, while a mutation that mimicks phosphorylation at Thr^{695} (T695E) created a constitutively desensitized receptor with respect to PLC activation [201].

These data strongly suggest that signalling pathways modulated by the mGlu1a receptor (and perhaps group I mGlu receptors generally) can be differentially regulated by receptor phosphorylation. Such data may provide a mechanistic explanation for a phenomenon reported for both presynaptic [134,202] and postsynaptic [203] group I mGlu receptors, whereby the coupling of the receptor to downstream effectors can be altered ('switched') by the pattern of receptor stimulation (Figure 4).

Involvement of GRKs in desensitization of group I mGlu receptors

Although PKC-mediated phosphorylation of group I mGlu receptors may be the dominant desensitization mechanism in some cell backgrounds, considerable evidence has accrued to suggest that this is not the only mechanism. Thus mGlu5 receptor desensitization can occur independently of PKC activity in astrocytes [107], and the desensitization of the mGlu1c receptor (assessed at the level of receptor phosphorylation and phosphoinositide turnover) caused by agonist is only partially recapitulated by phorbol ester addition [204]. Further, although both agonist and phorbol ester cause a marked decrease in mGlu1a-receptor-mediated phosphoinositide turnover in BHK cells, agonist-mediated desensitization is unaffected by PKC inhibitors [99].

Recent studies have shown that GRKs are directly involved in the phosphorylation and homologous desensitization of the mGlu1a receptor. To date, two different studies have reported on the recombinant co-expression of the mGlu1a receptor with GRKs in fibroblasts [205,206]. Both studies provide evidence for a role for GRKs in the modulation of mGlu1a receptor signalling and desensitization by agonist. However, the studies differ with respect to the GRK subtypes implicated in receptor regulation. Thus, while both studies indicate that expression of GRK2 ('β-adrenergic receptor kinase') and GRK5 significantly attenuates mGlu1a- and mGlu1b-receptor-mediated phosphoinositide hydrolysis, differing results have been reported with respect to overexpression of GRK4 and GRK6. Further demonstration of a physiological role for GRK4 in the control of mGlu1a receptor signalling was obtained by using GRK4 antisense constructs, which resulted in a significant amelioration of the agonist-induced desensitization, assessed with respect to the intracellular Ca^{2+} response [206]. The ability of GRK2 to inhibit mGlu1a signalling was correlated with its ability to increase mGlu1a phosphorylation upon exposure to agonist [205]. Although these studies clearly demonstrate that GRKs can affect mGlu1a receptor activity, further work is required to identify more definitively the GRK subtypes involved in the control of the group I mGlu receptors in the central nervous system.

A critical role for GRKs in the agonist-mediated internalization of the mGlu1a receptor has also been demonstrated recently by Sallese et al. [206], who showed that agonist-mediated internalization of the mGlu1a receptor in HEK 293 cells required co-expression of GRK4. These observations support a role for homologous receptor phosphorylation in the internalization process. In contrast with HEK 293 cells, cerebellar Purkinje cells can internalize endogenously expressed mGlu1a receptor by a mechanism that is impaired when GRK4 expression is suppressed using an antisense strategy. Agonist-mediated internalization of

mGlu1a receptors has also been analysed using a green fluorescent protein-tagged mGlu1a receptor expressed in HEK 293 cells [156]. In this model system, exposure to agonists resulted in a partial loss of the cell surface receptors. A lack of agonist-induced internalization of the mGlu1a receptor has also been reported in transfected BHK cells [207], whereas phorbol ester treatment was shown to induce the redistribution of receptors to endosome-enriched membrane fractions. Interestingly, both agonists and phorbol ester induced the internalization of the mGlu1b receptor splice variant. This may indicate that the long C-terminus of the mGlu1a receptor plays an important role in its homologous regulation. Together, these observations suggest that different mechanisms are involved in the internalization of group I mGlu receptors. Very recent studies have provided the first evidence that sequestration of these receptors occurs via a clathrin-, arrestin- and dynamin-dependent pathway [155].

In summary, group I mGlu receptors constitute potential substrates for both second-messenger-regulated protein kinases and GRKs, and receptor phosphorylation has been shown to affect functional coupling to G-proteins and downstream signalling pathways. However, in contrast with what is generally proposed for the vast majority of GPCRs, the phosphorylation of the mGlu receptor is not only involved in classical receptor desensitization. Phosphorylation of some critical residues also provides the possibility to control the functional coupling of the receptor with distinct intracellular proteins involved in signal transduction. More surprisingly, phosphorylation may also contribute to *decreasing* rapid receptor desensitization. Therefore homologous and heterologous receptor phosphorylation processes participate in both the negative and the positive regulation of group I mGlu receptor signalling.

FUTURE TRENDS

Considering that only 10 years have elapsed since the molecular cloning of the first mGlu receptor, enormous progress has been made with respect to group I mGlu receptor structure, signalling and regulation. From a pharmacological standpoint, recent advances by medicinal chemists have at last provided useful agents with which to evaluate mGlu1/5 receptor functions in the brain, and already the therapeutic potential of mGlu1- and mGlu5-receptor-selective antagonists is becoming clearer [208]. Of particular importance here is the development of non-competitive antagonists/inverse agonists with the potential to traverse the blood–brain barrier, which will allow mGlu receptor function to be manipulated and evaluated *in vivo*.

Despite this considerable progress, we are still at a very preliminary stage in our understanding of how the group I mGlu receptors fulfil their various roles within the brain. Although key protein scaffolds have been identified for mGlu1/5 receptors, which suggest mechanisms for targeting receptors to specific loci within neurons, there is still much that is unclear about the roles such proteins may play with respect to influencing the level of receptor expression and bringing about synaptic remodelling through the induction of non-cross-linking Homer proteins [63,144,209]. In this context it is interesting to note that immunocytochemical analyses of the subcellular localizations of the mGlu1 and mGlu5 receptors in dendrites of rat and monkey substantia nigral neurons have revealed marked differences in the proportion of group I mGlu receptors expressed at the plasma membrane compared with intracellular loci [210]. Hubert et al. [210] suggest that the high proportion of mGlu5 receptor immunoreactivity located intracellularly may represent a nascent pool of receptors that is available for recruitment by synaptic-activity-dependent mechanisms.

Although the use of recombinant/model systems has allowed many of the 'ground rules' regarding signalling potentials to be established, information on group I mGlu receptor signalling mechanisms in mature neurons remains scant. Accruing data suggest that cross-talk can occur between iGlu and mGlu receptors at synaptic specializations, which may contribute to the adaptative changes seen in synaptic efficacy in phenomena such as long-term potentiation and long-term depression. For example, co-operation between NMDA receptor activation and group I mGlu receptor activity can lead to 'supralinear' Ca^{2+} signalling [211,212]. This means that iGlu-receptor-mediated Ca^{2+}-influx and mGlu-receptor-mediated, $Ins P_3$-dependent Ca^{2+}-release mechanisms synergize to bring about a more marked increase in neuronal Ca^{2+} concentration, perhaps converting a localized dendritic rise in Ca^{2+} into a Ca^{2+} wave which travels along the dendrite and invades the cell body. Such 'supralinearity' may allow detection of coincident stimuli and propagation of Ca^{2+} waves to influence neuronal activity at the transcriptional level [213].

Other cross-talk mechanisms between iGlu and mGlu receptors may also be important in determining postsynaptic excitability. Thus bidirectional regulation has been shown to occur between NMDA and mGlu5 receptors (see [200]), with NMDA receptor activation modifying the time course of mGlu5 receptor desensitization (and potentially acute-agonist-stimulated oscillatory Ca^{2+} responses). Other pathways may also regulate group I mGlu receptor signalling; for example, tyrosine phosphorylation has been shown to affect both mGlu1 receptor and $G_{q/11}$ activation [214,215], perhaps allowing receptor and non-receptor tyrosine kinases to modulate mGlu receptor signalling at multiple pathway loci. Reciprocally, it has been shown recently that group I mGlu receptors can regulate the secretion of neurotrophins from hippocampal neurons [216].

Despite the glimpses afforded by studies over the past 10 years into the complexity of group I mGlu receptor neuronal signalling networks, a great deal has still to be learned. Future research will undoubtedly provide new insights, not only into the group I mGlu receptors in particular, but also the family C GPCRs generally.

We thank Dr Mark S. Nash and Julie V. Selkirk for their helpful comments and discussion, which have helped to shape this review. We gratefully acknowledge the Wellcome Trust, the National Fund for Scientific Research (F.N.R.S., Belgium; Convention FRSM 3.4588.00) and the Belgian Queen Elisabeth Medical Foundation for their financial support, and Professor S.R. Nahorski for his support and ongoing research collaboration. E.H. is a Research Associate of the F.N.R.S. (Belgium).

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