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Perspective

Metabotropic Glutamate Receptors: Novel Targets for Drug Development

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Introduction

Nerve cells communicate by releasing chemical substances at specialized structures called synapses, which can mediate excitation or inhibition of the target cell. At most excitatory synapses in the mammalian brain the released substance is the amino acid L-glutamate (L-Glu), which binds to and activates a variety of receptors. These glutamate receptors directly mediate or indirectly modulate synaptic transmission and, furthermore, also regulate the way in which electrical signals are integrated by nerve cells.

Excessive activation of glutamate receptors or disturbances in the cellular mechanisms that protect against the potential adverse consequences of physiological glutamate receptor activation have been implicated in the pathogenesis of a diverse group of neurological disorders. These disorders include epilepsy, ischaemia, central nervous system (CNS) trauma, neuropathic pain, and chronic neurodegenerative diseases. Over the last decade this hypothesis has stimulated intense interest in the development of compounds blocking activation of glutamate receptors.

Glutamate receptors are classified into ionotropic and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors form ligand-gated ion channels and, thereby, directly mediate electrical signaling of nerve cells. mGluRs indirectly regulate electrical signaling by influencing intracellular metabolic processes via G-proteins.

This perspective article focuses on the mGluR family. In particular, we discuss the structure of mGluRs and their functional properties in recombinant mammalian cells. We also report on the pharmacological properties of a variety of agonists and antagonists for mGluRs and their subtype specificity. Also discussed is the physiological role of mGluRs in the CNS and their possible involvement in pathophysiological processes. It will become apparent that mGluRs are equally promising as targets for drugs modulating glutamatergic synaptic transmission as are ionotropic glutamate receptors.

Discovery of mGluRs

The existence of mGluRs was first postulated in 1985 based on biochemical studies demonstrating that L-Glu stimulates inositol trisphosphate (IP_3) accumulation in the CNS.[1,2] This hypothesis was further supported by showing that a glutamate receptor coupled to phospholipase C (PLC) via G-proteins could be expressed in *Xenopus* oocytes following injection of total mRNA isolated from rat brain.[3] The molecular existence of a G-protein-coupled glutamate receptor was finally proven by functional expression cloning of a cDNA encoding the rat mGluR1a in the laboratories of Nakanishi and Mulvihill.[4,5]

Within the last three years a family of related mGluR cDNAs was isolated by cross-hybridization and PCR amplification. To date, eight different subtypes have been described, termed mGluR1 to mGluR8.[1,2,6-13] The majority of these mGluR subtypes are not coupled to phospholipase C but are instead negatively coupled to adenylate cyclase, at least when expressed in non-neuronal mammalian cells.

Structural and Functional Domains of Cloned mGluRs

The mGluRs form a new family of receptors, which is much larger in size and does not show any significant sequence similarity to other previously known members of the G-protein-coupled receptor family. However,

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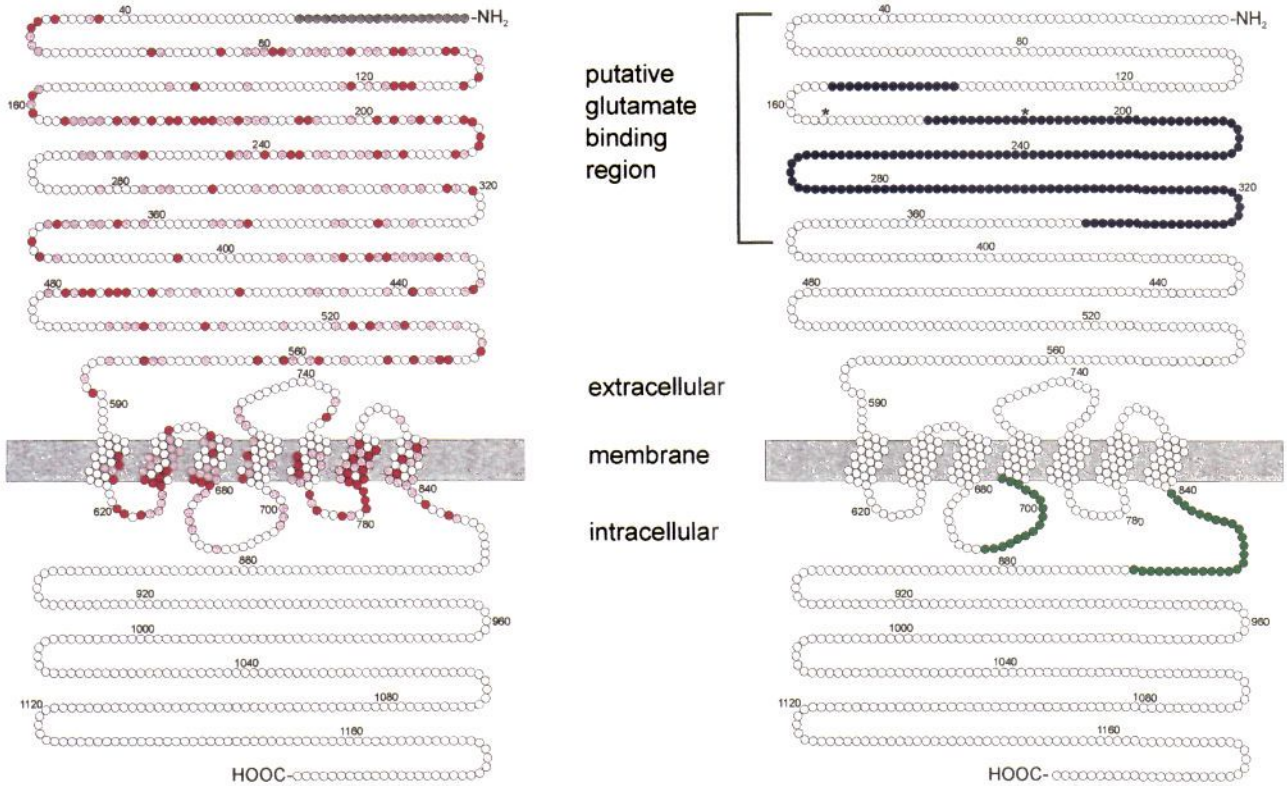

Figure 1. Structural features of mGluRs. (Left) Amino acids forming the extracellular, seven transmembrane, and intracellular domains of mGluR1a. Amino acids identical between mGluR1 to mGluR7 are drawn in red; amino acids identical in at least five of these seven mGluR subtypes are drawn in pink. The putative signal peptide sequence at the amino terminus is indicated in gray. (Right) Putative glutamate binding region[16] and regions proposed to determine specificity of G-protein coupling of mGluR1.[21] The putative glutamate binding regions is homologous to bacterial periplasmic binding proteins.[16] Amino acid sequences drawn in purple are recognized by antibodies blocking mGluR1 activation.[26] Point mutations at sites indicated by asterisk (*) result in a decrease of agonist binding affinity and impaired functional activation.[16]

mGluRs still reveal the conserved structural architecture of G-protein-coupled receptors. Each receptor displays a putative signal peptide at the amino terminus, suggesting that this domain is extracellular. Seven closely linked hydrophobic segments are predicted to form the seven transmembrane domains with three short intracellular and three extracellular loops separated by membrane-spanning segments. The carboxy terminus is thought to be intracellular. Figure 1 illustrates the principal structural features of mGluRs.

A sequence comparison of all mGluRs reveals 40−70% identity of amino acid sequences. On the basis of sequence similarity, mGluRs can be classified into three subgroups.[14] The sequence similarity of mGluR subtypes of the same subgroup is 67−70% and is reduced to 42−45% between members of different subgroups. Recently, a cDNA encoding a new member of the mGluR family has been isolated by expression cloning from a bovine parathyroid cDNA library. It encodes a receptor, termed PCaR1, that responds to changes in the extracellular concentration of specific ions such as Ca^{2+}. The deduced amino acid sequence of this "Ca^{2+}-detector" is of a similar size to mGluR1 and mGluR5 and shows about 25−30% sequence similarity with all members of the mGluR family.[15]

The highly conserved regions are the first and third intracellular loops and the transmembrane regions, which are thought to be involved in G-protein coupling and signal transduction. Twenty cysteine residues are conserved in all mGluRs and PCaR1, 19 of which are located in the extracellular domain and extracellular loops. Nine cysteine residues in the extracellular domain are located close to the first transmembrane region, similar to the cysteine-rich regions of tyrosine kinase receptors.[16]

The carboxy-terminal domain of the mGluR family is variable in length, and sequence conservation among all mGluRs is restricted to residues close to the seventh transmembrane region. Interestingly, splice variants for several mGluR subtypes including mGluR1,[6,17] mGluR4,[18] mGluR5,[19,20] and mGluR7 (Flor *et al.*, manuscript in preparation) are generated by alternative splicing in the carboxy-terminal domains. Evidence indicating that these splice variants exhibit different interactions with G-proteins[17,21−23] and are specifically expressed at the subcellular level[24] is accumulating.

The most conserved regions among the mGluRs and the Ca^{2+}-sensing PCaR1 are a hydrophobic segment and surrounding segments in the extracellular domain. It is postulated that these regions form the ligand binding site, based on the following lines of evidences: (i) Takahashi *et al.*[25] concluded from the analysis of a series of chimaeras between mGluR1 and mGluR2 that the N-terminal two-thirds of the extracellular domain of mGluR2 are sufficient to convert the agonist profile of mGluR1 into that of mGluR2. (ii) O'Hara *et al.* (1993) constructed a structural model of the extracellular domain of mGluRs on the basis of the weak sequence similarity between bacterial periplasmic amino acid binding proteins (PBPs) and the extracellular domain of mGluR1. Using the three-dimensional structures of several PBPs the model predicts that the glutamate

binding site is located in a cleft between two globular domains that are connected by a hinge region. It is proposed that binding of glutamate to one domain leads to a conformational change and trapping of glutamate in a pocket. This model is supported by mutational analysis, in which substitutions of Ser-165 or Thr-188 or both by alanine in the putative glutamate binding region strikingly reduced the affinity of mGluR1 for quisqualate and glutamate. (iii) Shigemoto *et al.*[26] generated two polyclonal antibodies against two regions of the extracellular domain of mGluR1, both of which inhibited functions mediated by mGluR1 in cultured Purkinje cells and chinese hamster ovary (CHO) cells expressing mGluR1. The regions used for the antibody production are included in the extracellular domain assumed to form the glutamate binding site.

This localization of the glutamate binding site is different from that of other G-protein-coupled receptors, such as catecholamines, which have their ligand binding site located in a pocket formed by the seven transmembrane domain segments.[27-29]

Functional Properties of mGluRs in Recombinant Mammalian Cells

In contrast to many other G-protein-coupled receptors, the existence of functionally distinct mGluRs has been established by molecular cloning and has not been primarily derived from pharmacological observations. As a consequence, the different mGluR subtypes were pharmacologically characterized in recombinant mammalian cell lines expressing a single cloned receptor. Thus, for each newly cloned subtype the signal transduction mechanism was elucidated first in recombinant cells by testing agonist induced effects on intracellular messengers. When expressed in CHO cells, mGluRs either positively couple to phospholipase C (PLC) or negatively couple to adenylate cyclase (Figure 2). Following characterization of the transduction pathway in recombinant cells, the rank order of potencies of several standard agonists was established for each mGluR subtype.

From these characterizations it was concluded that the mGluR subtypes can be classified into three groups based on signal transduction mechanisms and rank order of agonist potencies (Table 1).[14] Group I includes mGluR1 and mGluR5, which are coupled to PLC. mGluR1 and mGluR5 show very similar agonist selectivity, and both are potently activated by quisqualate.[4,5,30-32] In contrast, group II, which includes mGluR2 and mGluR3, and group III, consisting of mGluR4, mGluR6, mGluR7, and mGluR8, negatively couple to adenylate cyclase and, thereby, depress elevations in cAMP levels.[6,8,10-13] However, the agonist selectivity differs markedly between groups II and III. Group II effectively interacts with (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I) and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD), whereas group III potently interacts with (*S*)-2-amino-4-phosphonobutyric acid (L-AP4) and *S*-serine-*O*-phosphate (L-SOP).[6,14,33,34] Interestingly, this functional classification correlates well with similarities in the deduced amino acid sequences of the receptor proteins.

More recently, the human mGluRs have been cloned and expressed in mammalian cells.[35-38] In recombinant mammalian cells the transduction mechanisms and pharmacological properties of most subtypes are comparable to those described for the rat mGluRs. Table 2 summarizes effective concentrations for half-maximal responses (EC_{50} values) of several reference agonists at those mGluR subtypes for which such information is available.

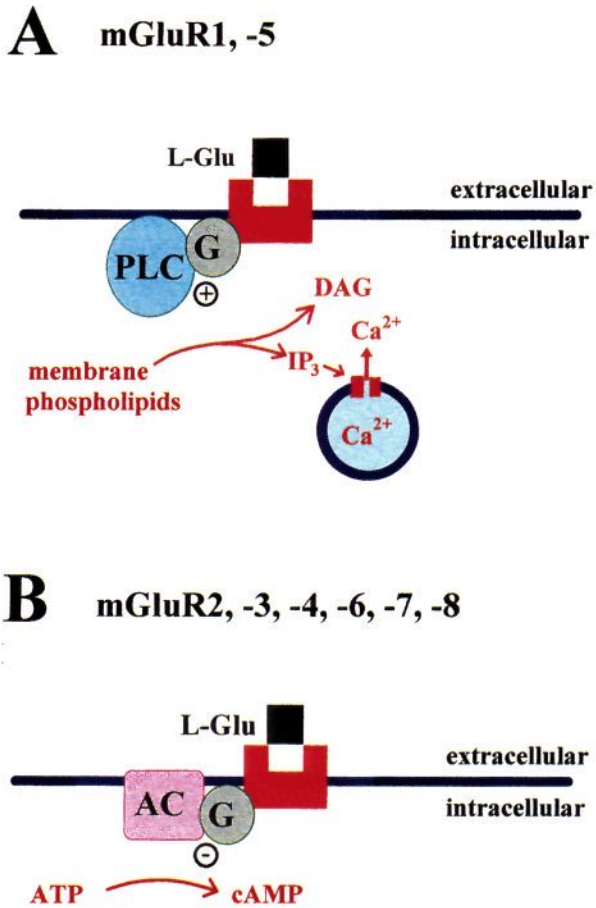

Figure 2. Signal transduction mechanism of mGluRs expressed in CHO cells. (A) Functional properties of class I mGluRs (mGluR1 and mGluR5) expressed in CHO cells. Binding of glutamate at the receptor protein results in a G-protein (G)-mediated activation of phospholipase C (PLC) and breakdown of membrane phospholipids into the chemical messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from internal stores. (B) Functional properties of class II and class III mGluRs (mGluR2, -3, -4, -6, -7 and mGluR8) expressed in CHO cells. Binding of glutamate at the receptor protein induces a G-protein-mediated inhibition of adenylate cyclase (AC). Depressed AC activity results in a reduced production of the chemical cyclic adenosine monophosphate (cAMP).

Chemistry of Agonists and Antagonists at mGluRs

The precise localization and the structural features of the transmitter recognition sites of mGluRs are not yet known. Therefore, no *ab initio* approach can be used for the design of new agonists and antagonists which is based on detailed knowledge of the physicochemical characteristics of the interaction between receptor protein and its ligands. Instead, the search for new ligands depends on the identification of novel lead compounds originating from screening and chemical efforts using established agonists and antagonists as structural templates.

Table 1

mGluR subtype	*amino acids*	*signal transduction*	rank order of potency of agonists	references
			Subgroup I	
mGluR1a	1199	↑ IP_3/Ca^{2+}	quisqualate > glutamate = ibotenate > (1*S*,3*R*)-ACPD	4,5,30,45
mGluR1b	906			23
mGluR1c	897			17
mGluR5a	1171	↑ IP_3/Ca^{2+}	quisqualate > glutamate = ibotenate > (1*S*,3*R*)-ACPD	7,20
mGluR5b	1203			20
			Subgroup II	
mGluR2	872	↓ cAMP	L-CCG-I > glutamate > (1*S*,3*R*)-ACPD > ibotenate ≫ quisqualate	6,45
mGluR3	879	↓ cAMP	glutamate > (1*S*,3*R*)-ACPD > ibotenate > quisqualate	11,45
			Subgroup III	
mGluR4	912	↓ cAMP	L-AP4 > glutamate > (1*S*,3*R*)-ACPD > quisqualate	11,45,108
mGluR6	871	↓ cAMP	L-AP4 > glutamate > (1*S*,3*R*)-ACPD ≫ quisqualate	8
mGluR7	915	↓ cAMP	L-AP4 ≫ glutamate > (1*S*,3*R*)-ACPD	10,12
mGluR8	908	↓ cAMP	glutamate > L-AP4	13

Table 2[a]

	agonist EC_{50} values								
receptor and species	L-Glu	Quis	*trans*-ACPD	Ibo	L-CCG-I	DCG-IV	L-AP4	L-SOP	references
mGluR1a (rat)	9	0.2	50	6					4, 30
	12	0.7	380	32					5
			22*						(#)
						>1000			45
mGluR1b (rat)	56	2.5	105	44					23
mGluR1b (human)	156	16.4	>1000*						(#)
mGluR1c (rat)	13	0.75	130	60			>1000		17
mGluR2 (rat)	4	>500	5	35			>1000		6
	11.8	>1000	7.7*		0.75		>1000		(#)
						0.3			45
mGluR2 (human)	4.7	>1000	12.1*		0.38		>1000		(#)
mGluR3 (rat)	3	40	8	10					11
						0.3			45
mGluR4a (rat)	5	>100	>100				0.5	4	11
	3.2	129	39	590			1.2		108
						>1000			45
mGluR4a (human)	20	≫500	166				0.2	1.3	(#)
mGluR5a (rat)	10	0.3	50	10					7
	8	0.2		8					20
mGluR5a (human)	4.1	0.15							(#)
mGluR5b (rat)	8	0.2		8					20
mGluR6 (rat)	16	1000	>200	1000			0.9	2.7	8
mGluR7 (rat)	1000						160	160	10
	1300	>1000	>1000				500		12
mGluR7 (human)	>500	>1000	>1000				200	<200	(#)
mGluR8 (mouse)	0.022	>100	>100	>100			0.4		13

[a] Concentrations for half maximal activation (EC_{50} values in 10^{-6} M) of mGluR subtypes, splice variants, and species homologues by glutamate (L-Glu), quisqualate (Quis), *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD), ibotenate (Ibo), (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I), (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), 2-amino-4-phosphonobutyric acid (L-AP4), and (*S*)-serine-*O*-phosphate (L-SOP). Values indicated by an asterisk (*) were obtained with (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid. References indicated by a pound symbol (#) are refs 35–38 and unpublished observations.

Agonists. Glutamate receptors are named according to the most prominent candidate as their physiological ligand; that is, the transmitter used to activate these receptors in the brain. While L-Glu not only activates mGluRs but also the ionotropic glutamate receptors, several structural derivatives of L-Glu act as more specific agonists (see above and Table 2). All these agonists share as partial structures an (*S*)-configurated α-amino acid group and an acidic functionality in the ω-position (see Figure 3).

It is not yet known how L-Glu interacts with the mGluR proteins and induces conformational changes enabling its interaction with G-proteins. Certain conformations of this acyclic neurotransmitter molecule seem to exclusively or preferentially recognize and bind to the various types of glutamate receptors.

In order to examine the conformational requirements of L-Glu for stimulating each L-Glu receptor subtype, Shimamoto *et al.*[39] synthesized four diastereomeric L-2-(carboxycyclopropyl)glycines as conformationally constrained analogues of L-Glu with varying configurations in positions 1′ and 2′. The pharmacological properties of these compounds are consistent with the hypothesis that mGluRs are activated by the extended form (see Figure 3) of L-Glu whereas the folded form stimulates ionotropic *N*-methyl-D-aspartate (NMDA) receptors. L-CCG-I, previously isolated by Fowden *et al.*[40] from immature fruits of *Aesculus parviflora* and *Blighia sapida*, proved to be the most potent mGluR agonist of the four diastereomers. DCG-IV,[41–44] a carboxylated analogue of L-CCG-I with (2*S*,1′*R*,2′*R*,3′*R*)-configuration and of considerable synthetic complexity, exhibited even higher agonistic potency for subgroup II mGluRs expressed in CHO cells with much lower affinity for mGluR1 or mGluR4.[45]

Antagonists. Phenylglycine derivatives were initially found to competitively antagonize (1*S*,3*R*)-ACPD-stimulated phosphoinositide (PI) hydrolysis.[46]

A more detailed characterization of the activities of phenylglycine derivatives has been performed using

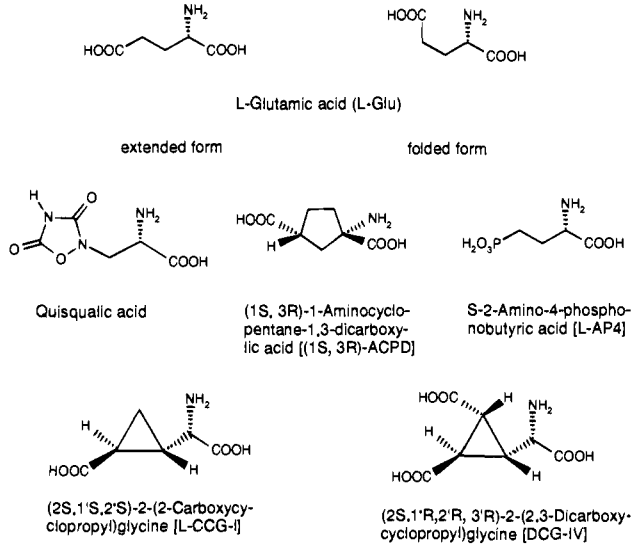

Figure 3. Structures of selected mGluR agonists.

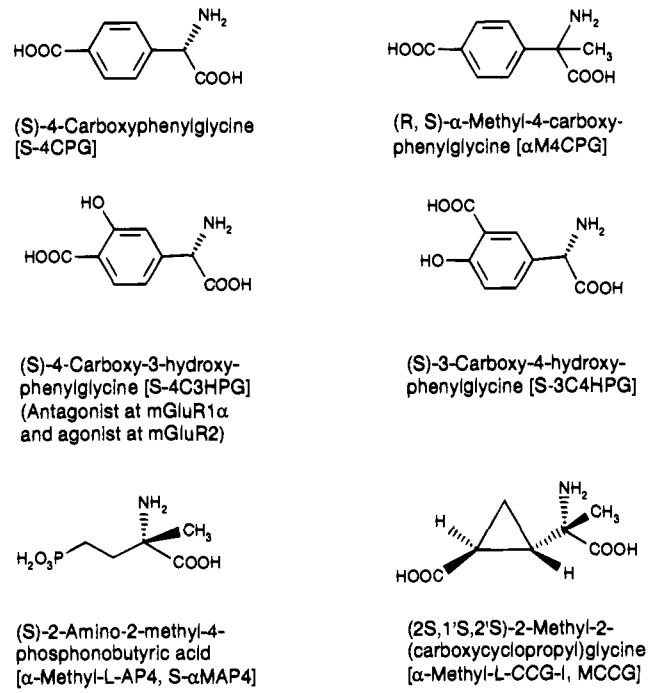

Figure 4. Structures of selected mGluR antagonists.

cloned mGluR1a, mGluR2, and mGluR4 stably expressed in baby hamster kidney (BHK) or CHO cells.[47,48] These studies revealed that the (*S*)-enantiomers of the phenylglycine analogues investigated bind preferentially to mGluRs and have little affinity for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors whereas (*R*)-4-carboxyphenylglycine and (*R*)-3-carboxy-4-hydroxyphenylglycine were confirmed as ligands for the competitive binding site of the NMDA receptor. (*S*)-4-Carboxyphenylglycine and its derivative (*R,S*)-α-methyl-4-carboxyphenylglycine are competitive antagonists at both mGluR1a and mGluR2 subtypes. The (+)-enantiomer of this α-methylated compound (absolute configuration not yet assigned) was substantially more effective than the (−)-antipode in reversing the effect of ACPD-induced inhibition of forskolin-stimulated formation of cAMP.[49] In contrast, (*R,S*)-4-carboxy-3-hydroxyphenylglycine and its (*S*)-enantiomer are potent competitive antagonists at mGluR1a and are potent agonists at mGluR2.[50] This example clearly illustrates the sensitive structure−activity relationship of this type of compounds which have to be further investigated for other mGluR subtypes.

Recently, α-methyl-L-AP4 (MAP4) and α-methyl-L-CCG-I (MCCG) were reported to show selective actions at L-AP4-sensitive and (1*S*,3*R*)-ACPD/L-CCG-I-sensitive receptors, respectively, on primary afferents to neonatal rat motoneurons.[51] However, the subtypes of mGluRs mediating these effects have not yet been established. The structures of mentioned antagonists are shown in Figure 4.

Thus, the relative steric positions of the functional groups and the configuration and conformational restriction of the amino acid functionality play an important role in providing specificity for agonists and antagonists not only for the three different classes of mGluRs but also for individual members. Interestingly, the mGluRs share no antagonists, so far, with other glutamate receptors.

Neurophysiological Role of Specific mGluR Subtypes

Historically, the first electrophysiological effects ascribed to mGluRs were induced by L-Glu or quisqualate, which are agonists at both metabotropic and ionotropic glutamate receptors. In these experiments, AMPA, kainate, and NMDA-receptor blockers were used in order to assure that a given effect of L-Glu (or quisqualate) was mediated by mGluRs.[52−56] This experimental design was overcome by the discovery that the racemic mixture of (1*S*,3*R*)- and (1*R*,3*S*)-ACPD (in the literature often termed *trans*-ACPD) acts as a specific mGluR agonist.[57] In the majority of systems, (1*S*,3*R*)-ACPD was found to be the active enatiomer.[58] On the basis of this knowledge, pharmacological studies have revealed a variety of effects mediated by mGluRs, including increased excitability and modulation of synaptic transmission.

mGluR-mediated increase in neuronal excitability has been well-investigated in hippocampal CA3 pyramidal cells where it results from the inhibition of two different potassium channels.[53] The first of these potassium channels is activated by membrane depolarization and generates a membrane current, termed I_M.[59,60] The second potassium channel is activated by a rise in intracellular [Ca^{2+}] and generates a current, termed I_{AHP}.[61] Excitation of the pyramidal cells involves depolarization of the membrane, opening of voltage dependent Ca^{2+} channels, and a consequent rise in intracellular [Ca^{2+}]. Depolarization and elevation of [Ca^{2+}] activate I_M and I_{AHP}, respectively, which counteract the excitation. The depression of these potassium currents via mGluRs removes this inhibition and, thereby, increases the excitability of the neuron.[53] This effect of L-Glu closely resembles that of classical neuromodulators such as acetylcholine (via muscarinic receptors),[62] noradrenaline,[63] or histamine.[64] Similar to the effect of muscarinic receptor activation, this effect in hippocampal pyramidal cells does not result from a release of Ca^{2+} from internal stores and is also not mediated by diacylglycerol, the soluble messengers produced by activation of mGluR1 or mGluR5.[53,65−67] Also, both I_M and I_{AHP} are not depressed by an decrease in cAMP concentration in these neurons, which is expected to occur

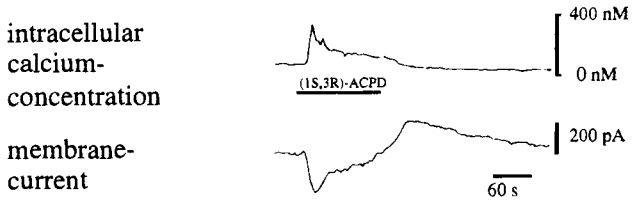

Figure 5. Functional properties of mGluR1 expressed in cerebellar Purkinje cells. Traces show time course of intracellular Ca^{2+} concentration and membrane potential. The mGluR was activated by (1*S*,3*R*)-ACPD.[83,84]

following activation of class II and III mGluRs.[60] Therefore, it seems likely that the coupling between mGluRs and the above-mentioned potassium channels is directly mediated by G-proteins or involves additional not yet established processes.

Synaptic transmission is modulated via mGluRs by several mechanisms including modulation of postsynaptic receptors and modulation of the amount of transmitter released into the synaptic cleft upon presynaptic stimulatory activity. Activation of mGluRs has been shown to modulate NMDA and AMPA receptor-mediated membrane currents in a variety of neurons most likely via phosphorylation of the respective ion channels.[68–71] Presynaptically, mGluRs reportedly depress release of L-Glu and GABA,[72,73] most likely via modulation of Ca^{2+} channels[52,74–76] mediating presynaptic influx of Ca^{2+} required for the release process.

While the above effects on synaptic transmission are reversible, evidence has accumulated that mGluRs are also involved in long-term regulation of synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy.[77–81] These phenomena serve as model systems for learning and memory, and accordingly, a role for mGluRs in these brain functions has been proposed.[82]

In most pharmacological studies, the specific mGluR subtype(s) involved remained undefined. More recently, evidence for the physiological role of distinct mGluR subtypes has been derived from studies using the more specific agonists (1*S*,3*R*)-ACPD, L-AP4, L-CCG-I, and DCG-IV and relating the agonist potency profiles in brain tissue with that obtained in recombinant cells. In several elegant studies the pharmacological identification of a mGluR subtype as the mediator in a specific neuronal signaling pathway was further substantiated by demonstrating local expression of the corresponding mRNA (*in situ* hybridization studies) or the presence of the receptor protein itself (immunocytochemical studies using subtype-specific antibodies).

In the following, neuronal responses mediated by well-defined mGluR subtypes are described. This list is not complete, and the mentioned expression of a specific subtype in a specific neuronal system does not imply that the same subtype is exclusively expressed there.

mGluR1. *In situ* hybridization studies and immunohistochemical analyses have demonstrated expression of mGluR1 in cerebellar Purkinje cells which lack mGluR5.[3,24] (1*S*,3*R*)-ACPD or quisqualate applied to cerebellar Purkinje cells induces a transient rise in intracellular Ca^{2+} concentration accompanied by a particular sequence of inward (excitatory) and outward (inhibitory) membrane currents[83,84] (Figure 5). These responses have been found not only *in vitro* in cerebellar slices but also *in vivo* in anaesthetized animals.[85] The agonist and antagonist profile of this excitation and the associated release of Ca^{2+} from internal stores strongly suggests that this response is mediated by mGluR1. In addition to these short-term effects, mGluR1 also seems to be involved in the mechanisms leading to LTD of transmission at the parallel fibre–Purkinje cell synapses.[26,86]

mGluR2 mRNA is found at high levels in the granule cells of the accessory olfactory bulb.[11,87] Immunohistochemical studies at the level of the electron microscope have revealed the specific localization of mGluR2 at dendrodendritic synapses between granule and mitral cells in the accessory olfactory bulb.[88] At these synapses, DCG-IV, a potent agonist for mGluR2, suppresses inhibitory GABAergic transmission via presynaptic mechanism. On the behavioral level, suppression of this GABAergic transmission by DCG-IV gates the formation of a specific olfactory memory.[45]

mGluR3 mRNA is expressed in glial cells and neurons.[11,87] The physiological function mediated by this subtype has been little investigated.

mGluR4 mRNA is most intensely expressed in the cerebellar granule cells which form synapses with Purkinje cells.[11] Synaptic transmission in this pathway is depressed by L-AP4 at a concentration which is indicative for a mediation by mGluR4 (Pisani and Knöpfel, unpublished observations).

mGluR5 is expressed in many brain areas and predominantly located at the level of dendrites and cell bodies of neurons (i.e., "postsynaptic" elements).[31,89]

mGluR6 is exclusively expressed in the outer zone of the inner nuclear layer of the retina, where ON-bipolar cells are located.[8,90] It has been suggested that mGluR6 stimulates a phosphodiesterase which catalyzes the breakdown of cGMP.[8] Due to cGMP-regulated cation channels, this results in a membrane hyperpolarization. Thus, mGluR6 has an important role in the synaptic transmission between photoreceptor cells and ON-bipolar cells in the visual system.[90,91]

mGluR7 mRNA is expressed at high levels in the cerebral cortex, the olfactory bulb, and cerebellar Purkinje cells.[10,12] It has been suggested that mGluR7 mediates inhibition of transmitter release at glutamatergic synapses in some brain regions.[12] In cerebellar Purkinje cells, mGluR7 is expressed in the cell body (Grandes *et al.*, unpublished observations) and mediates inhibition of P-type calcium channels.[92] Thus modulation of P-type calcium channels might be the underlying mechanisms for mGluR7-mediated inhibition of synaptic transmission.

mGluR8 expression has only recently been described by Duvoisin *et al.*[13]

Taken together, these diverse cellular responses mediated by mGluRs together with their distinct expression in different brain systems make them potential drug targets with a variety of possible mechanisms of action. In this context another point deserves to be noted. In some neuronal connections mGluRs mediate a functional inhibitory response such as depression of excitatory synaptic transmission and inhibitory membrane currents. Therefore, glutamate is not only an "excitatory" amnio acid, as often referred to, but can act also as an "inhibitory neuromodulator". As a consequence, agonists for at least some of the mGluR sub-

types should not be ignored as candidates for therapeutically effective drugs.

Interference of mGluRs with Pathophysiological Processes

A variety of diseases are thought to result from excessive activation of glutamate receptors or from a defect in the cellular mechanisms that protect against the potential adverse consequences of physiological glutamate receptor activation. These disorders include epilepsy, focal and global ischaemia, pain, and neurodegenerative diseases. The involvement of mGluRs in the pathophysiological processes underlying these diseases are reviewed in the following sections.

Epilepsy. Epilepsy can result from an increased glutamatergic transmission or a decreased GABAergic transmission.[93-96] There is a large body of literature on the possible therapeutic benefit of drugs inhibiting the activity of ionotropic glutamate receptors, but much less is known about the significance of mGluRs in epilepsy. There are several lines of argumentation supporting the hypothesis that drugs acting at mGluRs can serve in the treatment of epilepsy. As described above, class I mGluRs often mediate an excitation or increase excitability of neurons while class II/III mGluRs generally mediate a depression of synaptic transmission. On the basis of the premise that reduction of excitation or excitability is a useful therapeutic strategy for the treatment of epilepsy, antagonists for class I mGluRs and/or agonists for class II/III mGluRs might be antiepileptic. This concept has recently been supported by experimental findings showing that (*S*)-4C3HPG, an antagonist at mGluR1a receptors and an agonist at mGluR2,[47,97] antagonizes audiogenic-induced clonic and tonic convulsions in DBA/2 mice.[98]

Cerebral Ischaemia. Excessive action of glutamate can result in the death of neurons, a pathophysiological process underlying the concept of excitotoxicity. A key finding in this context was that exogenously applied glutamate or compounds selectively acting at specific glutamate receptor subtypes induced neurodegenerative changes in animal models reminiscent of pathological changes seen in human brain tissue following ischaemia.[99,100] In the past, much attention was given to drugs blocking activation of ionotropic glutamate receptors and in particular to NMDA receptors. More recently, mGluRs have also been postulated to be involved in excitotoxic mechanisms. There are several reports that (1*S*,3*R*)-ACPD applied to brain tissue can, on the one hand, induce neurodegeneration[101] *per se* but, on the other hand, can also attenuate glutamate-, NMDA-, or kainate-induced neurodegeneration.[102] These apparently contrasting observations might result from the fact that this compound is a potent agonist for both class I and class II mGluRs. Indeed, recently evidence is accumulating that more selective agonists for class II mGluRs such as DCG-IV and L-CCG-I potently protect neurons from NMDA- and kainate-induced degeneration.[103,104]

Pain. Noxious stimuli are transmitted to the spinal cord via C-fibers which form synapses in layers I and II of the dorsal horn at neurons which express mGluR5.[105] In line with these anatomical observations, electrophysiological experimentation has revealed an involvement of mGluRs in transmission of noxious signals in the spinal cord.[106,107] On the basis of these observations, it is tempting to speculate that mGluR5 is involved in pain transmission and drugs acting at this mGluR subtype might, therefore, be used in the treatment of chronic pain.

Neurodegenerative Diseases. Neurodegenerative diseases are associated with a heterogeneous group of pathophysiological processes some of which are of major clinical importance. The conceptual grounds for postulating a beneficial effect of agonists and/or antagonists for mGluRs in this area are overlapping with those discussed in the context of ischaemia.[104]

In addition to the therapeutic areas discussed above, mGluRs might be involved in diseases where pathophysiology cannot be directly associated with glutamatergic transmission. For instance, $GABA_A$ receptor-mediated neurotransmission is physiologically modulated via class II or class III mGluRs acting at GABAergic nerve terminals. Assuming that enhanced GABAergic neurotransmission results in a benzodiazepine-like systemic action, it can be speculated that class II or class III antagonists are of therapeutic benefit in the area of anxiety disorders. Since mGluRs couple to many different intracellular messenger systems, agonists and/or antagonists for these receptors might have potential therapeutic applications in neuropsychiatric diseases.

Conclusion and Outlook

The plethora of cloned receptor genes is not yet matched by a complete set of selective lead compounds. The potencies, selectivities, and bioavailabilities of the presently known agonists and antagonists for mGluRs are still limiting factors for biological research in the area of mGluRs. This is especially evident in the field of *in vivo* pharmacology. The discovery of more selective and potent mGluR agonists and antagonists than those currently available will be necessary to more completely establish the functions of specific mGluRs both in normal cell physiology and in pathological conditions.

The structural diversity of lead compounds is at present relatively limited and confined to amino acids. Additional variations of the available agonists and antagonists will have to be synthesized and precisely characterized for subtype selectivity before broader conclusions on structure–activity relationships can be drawn.

High-throughput screening of compound libraries using cells stably expressing individual receptors and measurement of functional effects is a promising approach for the identification of novel lead compounds which can be used as starting points for chemical optimization projects. This approach has the capacity to detect not only competitive agonists and antagonists but also modulators of yet undiscovered allosteric sites of the mGluRs.

The overall knowledge in this emerging field is still relatively scarce. Research in the field of mGluRs is expanding rapidly in many areas, including molecular biology, signal transduction, pharmacology, physiology, pathology, and medicinal chemistry. The development of more selective and more potent agonists and antagonists for mGluR subtypes is indispensable for rapid substantial progress in the mGluR area. The diversity of ligands recognized by other G-protein-coupled receptors suggests that a similar situation might also evolve

in this area. At present, the intense academic research efforts are already paralleled by active research programs in pharmaceutical companies. The availability of cells expressing individual recombinant receptors offers new perspectives for drug discovery, potentially leading to subtype-selective agonists/antagonists and, therefore, drugs with less side effects. New modulatory sites might also be detected with this approach. The potential to considerably increase basic knowledge about neuronal processes and to yield novel therapeutic agents for acute and chronic neurodegenerative diseases will continue to provide the driving force for continued research efforts in this new field.

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