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Metabotropic glutamate receptors: structure and new subtype-selective ligands

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Abstract

Metabotropic glutamate receptors (mGluRs) constitute an attractive target for the development of potential neuroprotective agents. Recent advances in the elucidation of the peculiar molecular architecture of mGluRs and in the design and synthesis of subtype selective ligands are discussed.

Keywords: Metabotropic glutamate receptors; Neuroprotection; Amino terminal domain; mGluR1 antagonists

1. Introduction

Metabotropic glutamate receptors (mGluRs) constitute a distinct family of G-protein-coupled receptors that respond to synaptically released L-glutamic acid, the major excitatory neurotransmitter in the CNS of vertebrates [1]. Pharmacological characterization and cDNA cloning have identified at least eight mGlu receptor subtypes, now classified into three groups on the basis of the signal transduction pathway and sequence homology (Table 1). Group I includes mGluR1 and mGluR5, which are positively coupled to the activity of phospholipase C (PLC) when expressed in heterologous systems, whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, mGluR8) are both negatively coupled to the activity of adenylyl cyclase (AC) but are endowed with a very different pharmacology.

In neurons, group I subtypes are almost exclusively localized at the post-synaptic terminal, where they increase the neuronal excitability and are involved in the potentiation of the glutamate-induced neurotoxicity [2]. This latter effect is associated with an enhanced function of the NMDA receptor, which is likely to result from an increased Src-mediated tyrosine phosphorylation mediated by the activation of mGluR1 (but not mGluR5) [3].

Group II and group III subtypes are prevalently localized at presynaptic terminals, away from the active area (with the exception of mGluR7, localized in the core of the active space). A post-synaptic and glial localization for group II subtypes has also been observed. According to their pre-synaptic localizations, group II and group III receptor subtypes are mainly involved in the inhibition of the transmitter release; hence, a general depression of the glutamatergic activity can be expected from their activation [2].

The molecular and the functional diversity of mGlu receptors, and the important therapeutic opportunities associated with their modulation, have fostered an intense research activity aimed at the discovery of potent and selective agonists, antagonists and allosteric modulators. The combined approach of rational drug design

Table 1
Classification of metabotropic glutamate receptors

Group I	mGluR1 mGluR5	↑PLC
Group II	mGluR2 mGluR5	↓AC
Group III	mGluR4 mGluR6 mGluR7 mGluR8	↓AC

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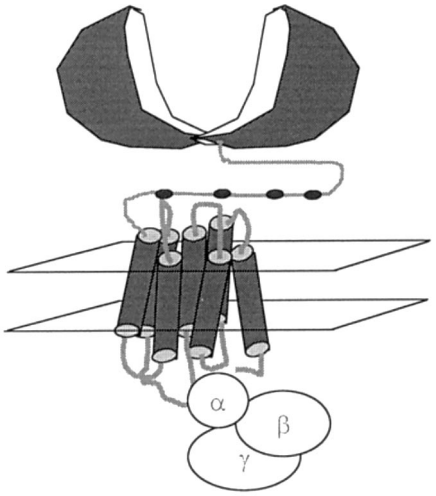


Fig. 1. Architecture of mGluRs.

and chemical library screening has allowed subtype-selective, nanomolar potent modulators to be discovered and characterized [1]. In turn, this has greatly improved our knowledge on the structure of mGlu receptors and their physiopathological role in a variety of brain diseases and disorders.

In this paper, we will focus our attention on structural aspects of mGluRs that are relevant for the understanding of the binding mode of agonists and competitive antagonists and on some recent advances in the medicinal chemistry of group I mGluRs.

2. Structure of mGlu receptors

Since their first cloning, it has become apparent that mGlu receptors constitute a family of G-protein-coupled receptors different from that of 'classical' GPCRs [4]. Nowadays, mGlu receptors are classified in the family C of GPCRs, together with vomeronasal pheromone receptors, taste receptors, Ca^{2+}-sensing receptors, $GABA_B$ receptors and the newly cloned retinoic acid inducible orphan receptors [5]. Even though the classical GPCR's macroarchitecture is conserved in mGlu receptors (i.e. the heptahelix transmembrane domains, an intracellular carboxy terminus and an extracellular amino terminus), several structural features are distinctive of this family. In particular, the coupling with the G-protein takes place at the level of the second and not the third intracellular loops; the seven transmembrane segments bear no sequence similarity with classical GPCRs; and, most importantly, the amino terminal domain is unusually extended (500–600 amino acids). A variety of pharmacological and biochemical evidence indicates that the ATD of mGluRs contains the binding site for the neurotransmitter and is sufficient to confer agonist and competitive antagonist selectivity [6,7]. In addition, truncated mGluR1 [8] or mGluR4 [9] receptors, including only the soluble extracellular amino terminal portion, maintain the agonist/antagonist binding characteristics of the wild-type receptors, thus indicating that the transmembrane region and the extracellular loops are not implicated in either the ligand binding or the preservation of a correct folding (Fig. 1).

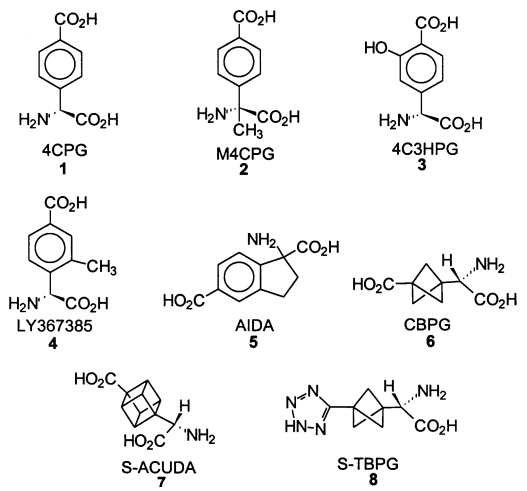
In 1993, O'Hara et al. proposed that the ATD of mGluR1 is homologous to Leu/Iso/Val/Binding Protein (LIVBP) [7], a member of the family of periplasmic binding proteins. On the basis of such sequence homology, it has been proposed that the ATD of mGluRs is composed of two globular domains, separated by a three-filament hinge region. A conformational equilibrium between an open (inactive) form, in which the two lobes are separated, and a closed (active) form, in which the two lobes are in close contact, has also been proposed. The sequence homology between mGluR subtypes, LIVBP, and other family C receptors, suggests the existence, confirmed in many cases by site-directed mutagenesis, of a core of conserved residues that are responsible for agonist binding [10]. These include Ser165, Thr188 and Arg78 (mGluR1 numbering). The presence of such a triad of evolutionarily conserved amino acids is, however, unable to provide an explanation for the observed subgroup- and subtype-selectivity displayed by many agonists. The origin of the selectivity must, therefore, be searched for in those areas of the binding pockets characterized by evolutionary divergences. Recently, allosteric modulatory sites on mGluR1, mGluR5 and mGluR2 subtypes have been identified, representing a promising target for the design of structurally novel mGluR ligands [11].

3. Search for mGluR1-selective antagonists

Evidence has accumulated in recent years to support the notion that group I mGluRs and the mGluR1 subtype in particular, are implicated strongly in the generation and propagation of neuronal damage following excitotoxic insult [12]. Possible mechanisms by which mGluR1 is thought to mediate the propagation of the excitotoxic stimulus include the direct mobilization of the intracellular calcium through IP_3 and DAG or the increased NMDA function achieved by Src tyrosine kinases mediated phosphorylation [3]. Regardless of which mechanism is predominant, mGlu1 antagonists have been proven effective in several models, in vitro, ex vivo and in vivo, of cerebral ischemia [13]. This observation has fostered the search for potent and selective mGluR1 antagonists. The class of carboxyphenylglycines (CPGs, **1**–**3**, Scheme 1), first reported by Watkins and coworkers in 1993 [14], has provided the source for the first mGlu receptor antago-

nists; nonetheless CPGs of the first generation were generally endowed with low potency and poor selectivity. Several research laboratories have since then addressed the problem of improving the potency and the selectivity of the CPG class of compounds. As a result, CPG derivatives such as **4**, endowed with low- to sub-micromolar range potency and group or subtype selectivity are now available. In the frame of a research project devoted to the design and synthesis of novel mGluR ligands, we addressed ourselves to the definition of the S.A.R. for the class of CPGs with the aim of disclosing the structural features responsible for selective antagonism towards individual mGlu receptor subtypes. As a first approach, we took into account the rotatable bond between the aromatic ring of 4-CPG (**1**) and the glycine moiety. The rigidification of such a bond into a five-membered ring yielded AIDA (**5**), a conformationally constrained analog endowed with a lower potency than the parent compound 4-CPG (**1**) but with an increased selectivity, with no activity at group II, group III and mGlu5 receptor subtypes [15].

The second structural feature to be investigated was the role played by the aromatic ring of CPGs. Indeed, we thought that the aromatic ring might either serve as a spacer between pharmacophoric groups or be involved in more specific interactions. Thus, we designed and synthesized (*S*)-CBPG (**6**) [16] and (*S*)-ACUDA (**7**) [17], where the propellane and the cubane moiety, respectively, substitute for the aromatic ring in keeping the pharmacophore groups in a co-planar, linear disposition but which are endowed with very different physico-chemical profiles. The good activity of **6** as an mGlu1 receptor antagonist was the confirmation that the aromatic ring is not strictly necessary for the activity. When tested as mGlu ligand, (*S*)-ACUDA (**7**) was shown to be only a weak mGlu1 antagonist. The loss of affinity of **7** with respect to 4-CPG (**2**) or *S*-CBPG (**6**) must, therefore, be ascribed to the increase in the volume of the spacer. It should be noted, however, that *S*-CBPG (**6**) and (*S*)-ACUDA (**7**) are also characterized by different distances between pharmacophoric groups. Indeed, since *S*-CBPG (**6**) is significantly shorter than (*S*)-ACUDA (**7**) and 4-CPG (**2**) itself, the structure–activity relationship study requires the effect of the distance between pharmacophoric groups to be investigated in the case of propellane derivatives. Thus, we have envisaged the synthesis of a novel propellane derivative, 2-(3′-(1H-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (*S*-TBPG, **8**) [18]. When tested as an mGluR ligand, *S*-TBPG (**8**) was shown to be a moderately potent but selective mGluR1 antagonist, with a potency approximately fourfold less than *S*-CBPG (**6**).



Scheme 1.

4. Conclusions

The results reported here indicate that modifications either at the spacer moiety or at the distal acidic group can modulate the activity as mGluR1 antagonists. Among the several derivatives synthesized and tested, *S*-CBPG (**6**) is the optimal one in terms of potency and selectivity, but the data collected so far indicate that further chemical manipulations may result in compounds endowed with improved biological profiles.

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