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Discovery and characterization of non-competitive antagonists of group I metabotropic glutamate receptors

Fabrizio Gasparini *, Philipp Floersheim, Peter Josef Flor, Micheline Heinrich, Werner Inderbitzin, David Ott, Adriana Pagano, Christine Stierlin, Natacha Stoehr, Ivo Vranesic, Rainer Kuhn

Novartis Pharma AG, Nervous System Research, K-136.694, Postfach, CH-4002 Basel, Switzerland

Abstract

We have investigated the mechanism of inhibition of the new group I mGluR antagonists CPCCOEt and MPEP and determined that both compounds have a non-competitive mode of inhibition. Furthermore using chimeric/mutated receptors constructs we have found that these antagonists act at a novel pharmacological site located in the trans-membrane (TM). Specific non-conserved amino acid residues in the TM domain have been identified which are necessary for the inhibition by CPCCOEt and MPEP of the mGlu1 and mGlu5 receptors, respectively. Using molecular modeling a model of the TM domain was built for both mGlu1 and mGlu5 receptor subtypes. Docking of CPCCOEt and MPEP into their respective model allowed the modelisation of the novel binding site. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: Metabotropic glutamate receptors; Molecular modeling; Non-competitive antagonists

1. Introduction

L-Glutamate is the main excitatory neurotransmitter in the mammalian brain and it acts through a heterogeneous family of two major types of receptor: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are ligand gated channels, whereas metabotropic receptors are G-protein-coupled receptors (GPCRs) linked to second messenger pathways. To date, eight metabotropic receptor subtypes have been identified and classified into three groups according to their amino acid identities, second messenger coupling and pharmacology [1]. Group I receptors (mGluR-1 and -5) are coupled to the phosphoinositide/Ca^{2+} cascade. Group II receptors (mGluR-2 and -3) and group III receptors (mGluR-4, -6, -7 and -8) are negatively coupled to adenylate cyclase. mGlu receptors are members of the superfamily 3 of GPCRs and possess a unique structure with a large extracellular N-terminal domain involved in the glutamate recognition site (Fig. 1) [2,3].

A variety of antagonists at the group I mGlu receptors have been described and these can be classified into two categories according to their mode of inhibition. Competitive antagonists such as (*RS*)-3-[1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]cyclobutanecarboxylic acid (LY357366) and (+)-2-methyl-4-carboxyphenylglycine (LY367385) (Fig. 2) [4] are amino acid derivatives that interact at the glutamate binding site located in the large extracellular N-terminal domain (Fig. 1). Recent advances have elucidated two novel compound classes with a non-competitive mode of inhibition and no structural analogies to amino acids (Fig. 2): the first class of antagonists is exemplified by (−)-2-hydroxyimino-1a,2-dihydro-1*H*-7-oxa-cyclopropa[b]naphthalene-7a-carboxylic acid ethyl ester ((−)-CPCCOEt) [5,6], and is selective for the mGlu1 receptor; the second class is exemplified by 2-methyl-6-(phenylethynyl)-pyridine (MPEP) [7], and is selective for the mGlu5 receptor (Fig. 2).

2. Characterization of the binding domain of non-competitive antagonists

Schild analysis indicated that CPCCOEt and MPEP act in a non-competitive manner by decreasing the

* Corresponding author.

E-mail address: fabrizio.gasparini@pharma.novartis.com (F. Gasparini).

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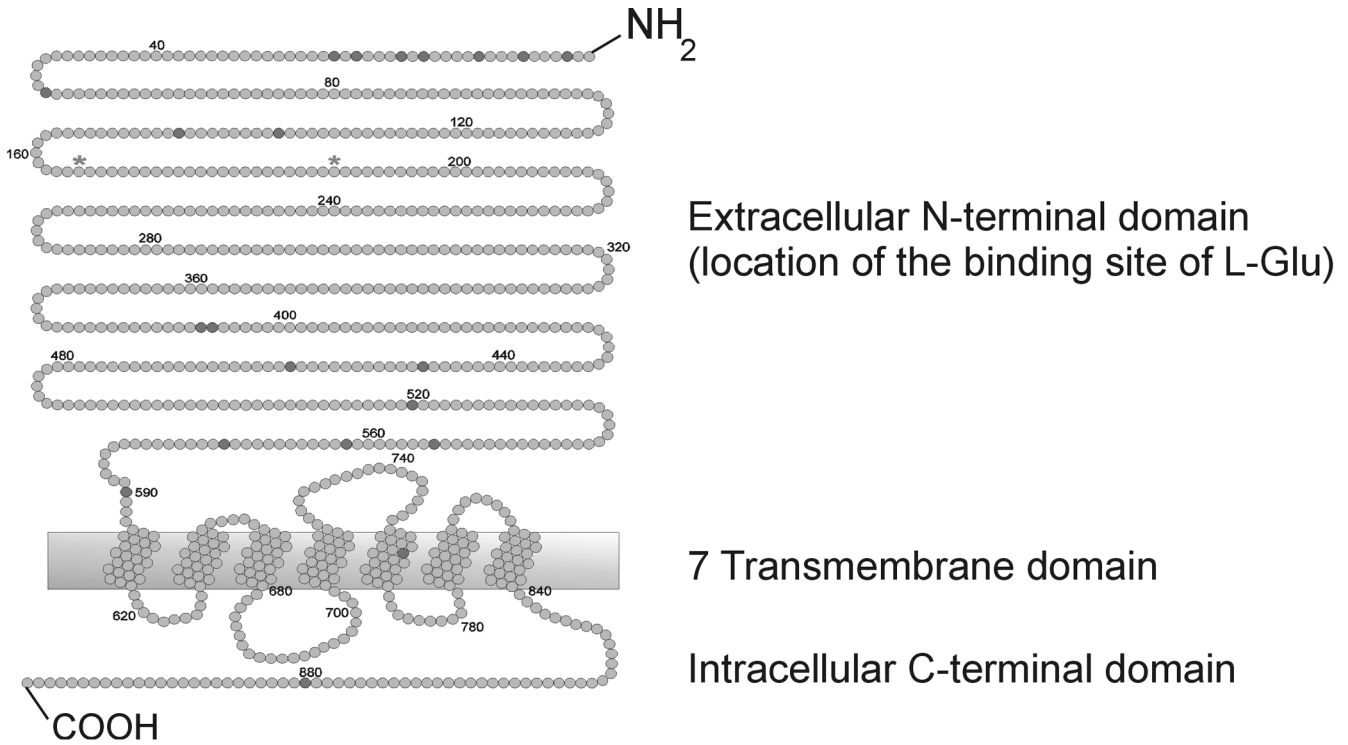


Fig. 1. Schematic representation of the mGlu receptors.

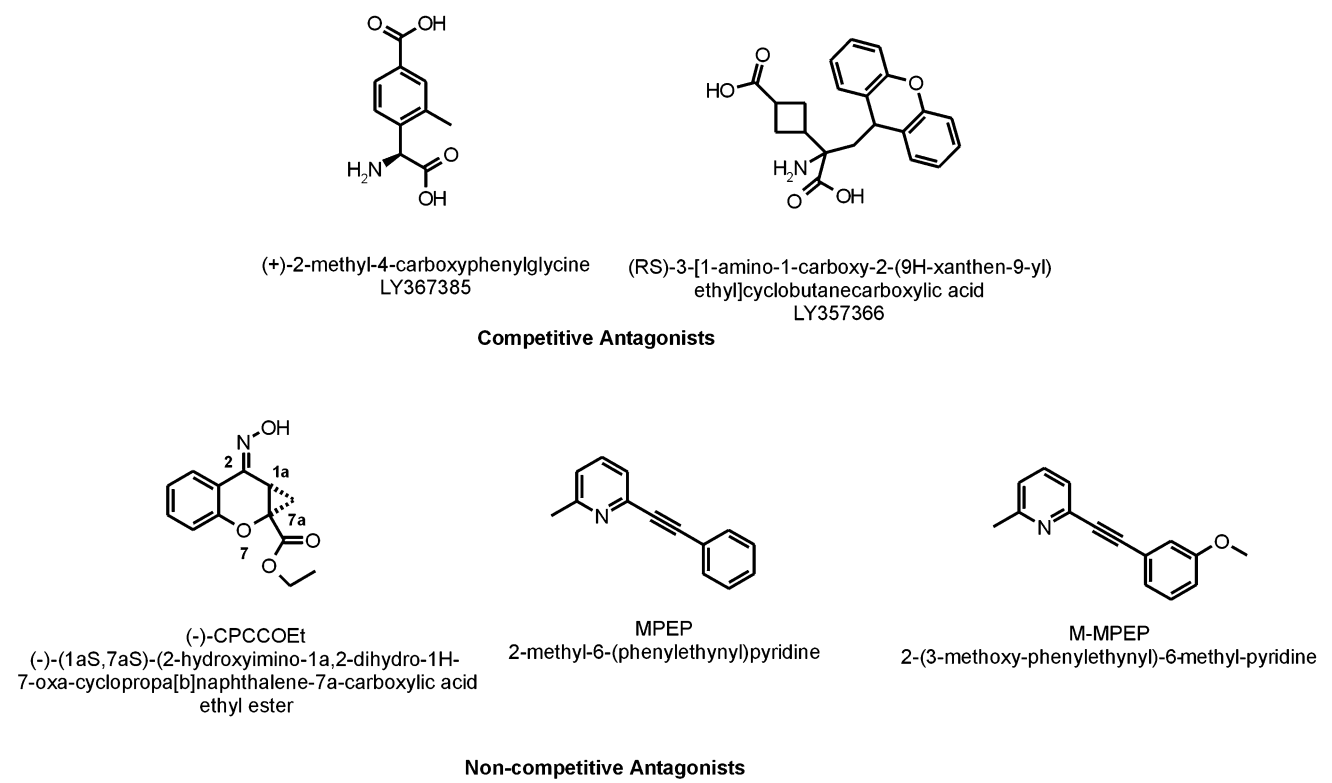


Fig. 2. Chemical structures of competitive and non-competitive group I antagonists.

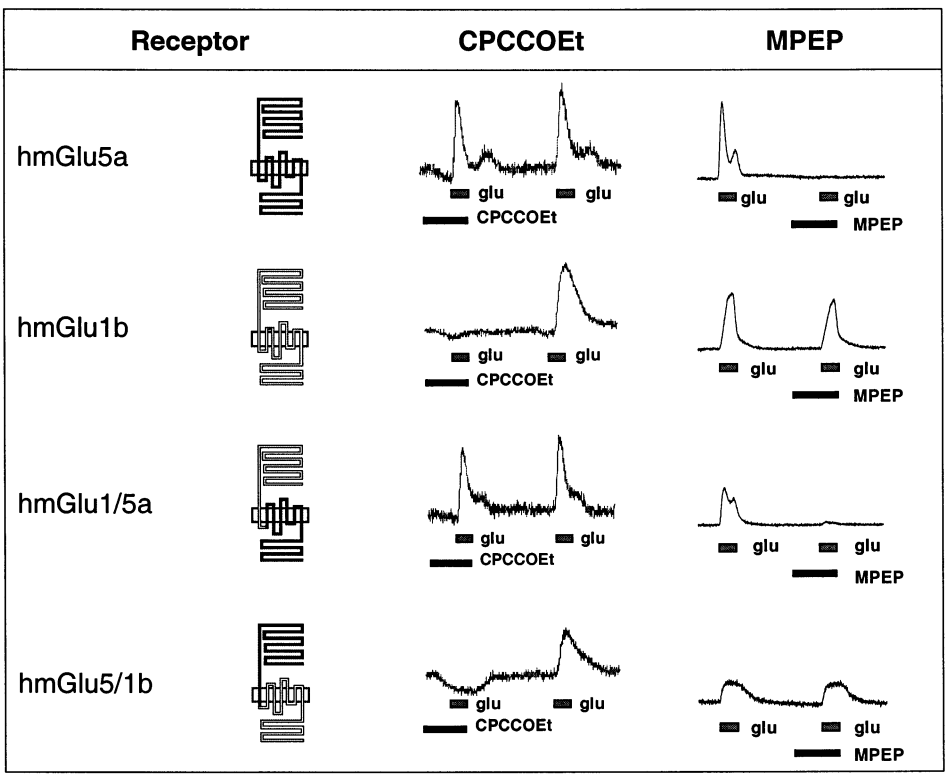
efficacy of glutamate-stimulated phosphoinositide hydrolysis without affecting the EC_{50} value of glutamate [8,9]. The non-competitive mode of inhibition suggests that the two compounds interact at a different site than the glutamate binding site located in the large extracellular domain. To elucidate the site of action, we have generated a set of chimeric hmGluR-1/5a and -5/1b receptors fused at the border between the large N-terminal extracellular domain and the first TM segment. These chimeric receptors were transiently expressed in COS cells and were shown to couple to PI turnover and a subsequent release of $[Ca^{2+}]_i$ from internal stores. Investigation of the inhibition by either CPCCOEt and MPEP showed that the respective transmembrane domains of the mGlu1 or the mGlu5 receptors are necessary to mediate the inhibitory activity (Table 1) [8,9].

To identify the amino acid residues mediating the selective receptor/ligand interaction, a series of chimeric receptors and single amino acid mutations was constructed. The inhibitory activity of CPCCOEt was studied using glutamate-induced Ca^{2+} responses, whereas for MPEP a displacement assay using the novel mGlu5 receptor radioligand [^{3}H]-M-MPEP (2-(3-methoxyphenylethynyl)-6-methyl-pyridine), a close analog of MPEP, was used. Substitution in the mGlu5 sequence of Ala810 in TMVII or Pro655 and Ser658 in TMIII with the homologous residues of hmGluR1 abolished [^{3}H]-M-MPEP binding [9]. Substitution in the mGlu1 sequence of Thr815 and Ala818, located at the extracellular surface of TMVII, with the homologous amino acids of hmGluR5a eliminated CPCCOEt inhibition of hmGlu1b [8]. In contrast, introduction of Thr815 and Ala818 at the homologous positions of hmGlu5a conferred inhibitory activity to CPCCOEt ($IC_{50} = 6.6$ μM, PI turnover assay), i.e. a gain-of-function and affinity ($IC_{50} = 3.5$ μM, [^{3}H]-M-MPEP displacement). These results indicate that MPEP and CPCCOEt bind to overlapping binding pockets in the TM region of group I mGluRs but interact with different non-conserved residues of mGluR1 and mGluR5, respectively (Fig. 3).

3. Molecular modeling

To suggest a plausible binding mode of CPCCOEt and MPEP, we have built molecular mechanics models of the putative seven TM domain of hmGlu1 and hmGlu5 based on the α-carbon template of the TM helices of rhodopsin [10]. To these models, CPCCOEt and MPEP were docked and the resulting complexes were energetically minimized using the program X-Plor [11]. The receptor docking model for mGlu1/CPCCOEt

Table 1
Inhibitory activity of CPCCOEt and MPEP on the mGlu1, mGlu5 receptors and the chimeric receptor constructs mGlu1/5 and mGlu5/1. Receptor constructs were transiently expressed in COS1 cells and tested for inhibition of glutamate-induced $[Ca^{2+}]_i$ responses



suggests that the amino acid residue Thr815 (TMVII) comes in close contact with the oxime OH of CPCCOEt [6]. In analogy, the model for mGlu5/MPEP suggests close contacts with the amino acid residues Ala810 (TMVII), P655 (TMIII) and S658 (TMIII) [9]. Furthermore, a comparison of the models suggests a partial overlap of the binding sites in the region of the pyridine ring of MPEP and the phenyl moiety of CPCCOEt. Other parts of these antagonists do not overlap, and suggest interactions with different TM helices (Fig. 4).

4. Conclusions

The pharmacological results obtained with the chimeric and mutated receptors have identified non-conserved amino acid residues that are essential for the inhibitory activity of CPCCOEt and the binding affinity of MPEP, respectively. Our docking models show that the allosteric binding site is formed by the upper part of the TM helices III, VI and VII for the hmGlu1 receptor and by TMII, III, VI and VII for the hmGlu5 receptor. This suggests that the novel

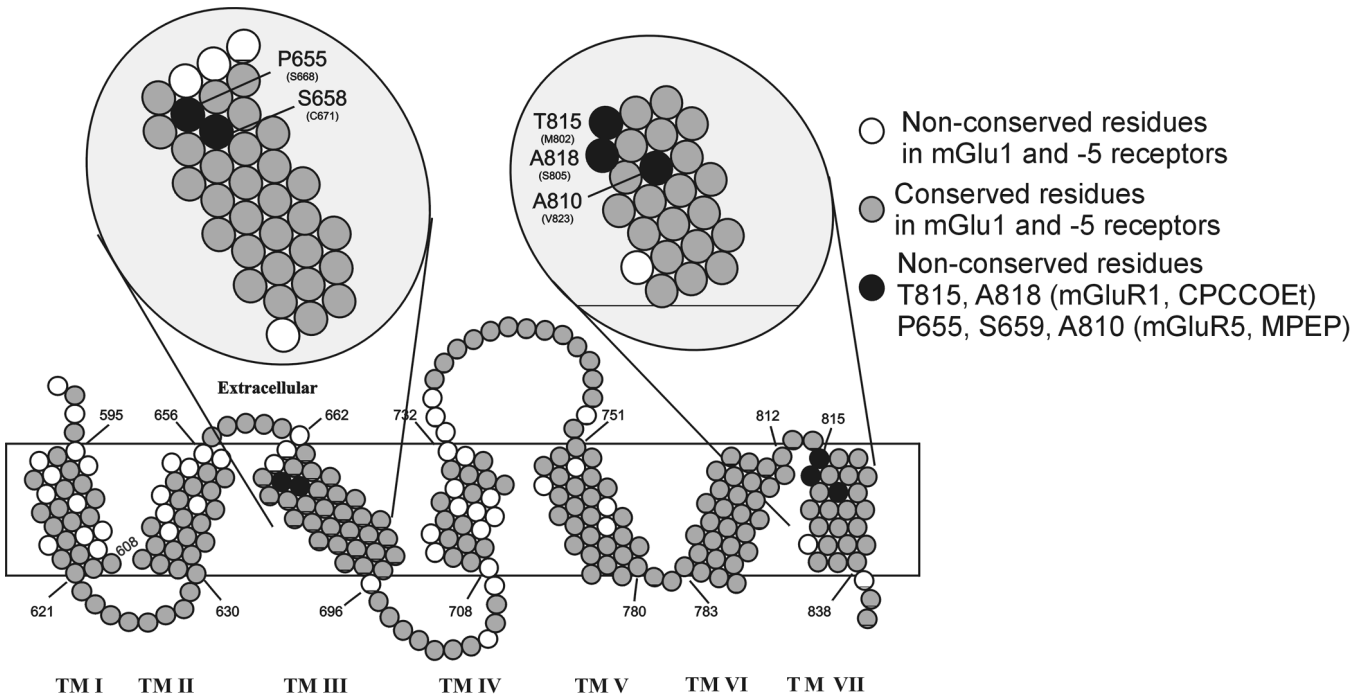


Fig. 3. Schematic representation of the TM domain. Non-conserved amino acid residues between mGlu1 and mGlu5 are shown in white. Non-conserved amino acid residues mediating the inhibitory activity of CPCCOEt (T815, A818, TMVII) and of MPEP (A810, TMVII; P655, S658, TMIII) are shown in black.

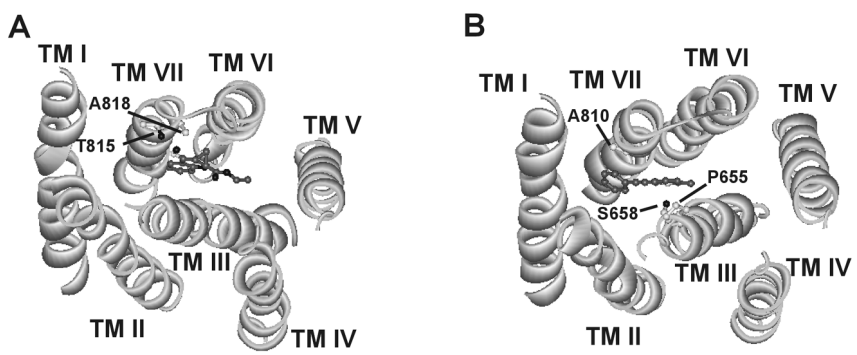


Fig. 4. (A) Model of (−)-CPCCOEt (shown with dark gray carbon atoms, nitrogen in light gray and oxygen atoms in black) docked to the TM model of the hmGlu1 receptor. Closely interacting amino acid residues are highlighted (carbon atoms in light gray, oxygen in black) (T815, A818,TMVII). (B) Model of MPEP (shown with dark gray carbon atoms, nitrogen in white) docked to the TM model of the hmGlu5 receptor. Closely interacting amino acid residues are highlighted (carbon atoms in light gray, oxygen in black) (A810, (TMVII); P655, S658 (TMIII)).

allosteric binding sites for the non-competitive antagonists CPCCOEt and MPEP are homologous to the binding site of antagonists at other GPCRs, such as the adenosine or serotonin receptors [12,13].

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