Pharmacological characterization of (4R)-alkyl glutamate analogues at the ionotropic glutamate receptors - Focus on subtypes iGluR5-7
Lennart Bunch, Thierry Gefflaut, Sébastien Alaux, Emmanuelle Sagot, Brigitte Nielsen, Darryl S. Pickering

To cite this version:
Lennart Bunch, Thierry Gefflaut, Sébastien Alaux, Emmanuelle Sagot, Brigitte Nielsen, et al.. Pharmacological characterization of (4R)-alkyl glutamate analogues at the ionotropic glutamate receptors - Focus on subtypes iGluR5-7. European Journal of Pharmacology, Elsevier, 2009, 609, pp.1-4. hal-00376953

HAL Id: hal-00376953
https://hal.archives-ouvertes.fr/hal-00376953
Submitted on 20 Apr 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Pharmacological characterization of (4R)-alkyl glutamate analogues at the ionotropic glutamate receptors — Focus on subtypes iGlu5–7

Lennart Buncha, Thierry Gefflautc, Sebastien Alauxc, Emanuelle Sagotc, Birgitte Nielsena and Darryl S. Pickeringb

aDepartment of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark
bDepartment of Pharmacology and Pharmacotherapy, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark
cLaboratoire SEESIB, UMR 6504, Département de Chimie, Université Blaise Pascal, 63177 Aubière Cedex, France

Abstract

The kainic acid (kainate, KA) receptors belong to the class of ionotropic glutamate (iGlu) receptors in the central nervous system. Five subtypes have been identified, which have been termed KA1,2 and iGlu5–7. In the search for subtype selective ligands, α-amino-5-tert-butyl-3-hydroxy-4-isoxazolyl)propionic acid (ATPA), (4R)-methyl Glu (1a), and E-4-neopentylidene Glu (2f) have all previously been reported as selective agonists for the iGlu5 receptor subtype. In this paper, we present the pharmacological evaluation of a five-compound series of (4R)-alkyl Glu analogs (1b–e, g) which may be envisaged as conformationally released designs of ATPA and 4-alkylidenes 2a–h. Most notable is the pharmacological profile for (4R)-isopentyl Glu (1g) which shows a 10-fold increase in binding affinity for the iGlu5 receptor subtype (Ki = 20.5 nM) in comparison with its E-4-alkylidene structural isomer 2g. Furthermore, 1g displays high selectivity over other KA receptor subtypes (KA 1,2 and iGlu 6,7), AMPA-, and NMDA receptors (2050 and > 5000 fold, respectively).

Keywords: Glutamate receptor; Kainate receptor; Subtype selectivity; iGlu5; iGlu6; iGlu7

1. Introduction

(S)-Glutamic acid (glutamate, Glu) is the major excitatory neurotransmitter in the central nervous system (CNS) activating the plethora of ionotropic Glu (iGlu) receptors and metabotropic Glu (mGlu) receptors ([Bräuner-Osborne et al., 2000] and [Meldrum, 2000]). Whereas the iGlu receptors are ion channels and thus mediate a fast excitatory response (Na+, K+, Ca2+ flux), the mGlu receptors are classified as G-protein coupled receptors and generate a slower signal transduction through second messenger systems. On the basis of pharmacological studies, the iGlu receptors are further divided into: 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors (homo- or heteromeric receptors comprising the subunits iGlu1–4), kainic acid (KA) receptors (homo- or heteromeric receptors comprising the subunits iGlu5–7 and KA1,2) (Wisden and Seeburg, 1993), and the N-methyl-d-aspartic acid (NMDA) receptors (heteromeric receptors comprising the subunits NR1, NR2A–D, NR3A–C). The mGlu receptors comprise eight homodimeric subtypes, mGlu1–8, which are grouped with respect to the second messenger system activated, pharmacology, and molecular biology (group I: mGlu1,5 receptors; group II: mGlu2,3 receptors; group III: mGlu4,6–8 receptors) (Ferraguti and Shigemoto, 2006). Termination of the excitatory signal is controlled by uptake of Glu from the synaptic cleft by the excitatory amino acid transporters (EAATs) (Beart and O'Shea, 2007). To date, five subtypes have been identified of which EAAT1–3 are high capacity Glu transporting proteins, EAAT4 functions predominantly as a chloride ion channel and EAAT5 is present exclusively in the retina.

In order to study the function of a single receptor subtype, when found in its intrinsic biological environment, the application of subtype selective ligands (agonists, partial agonists or antagonists) is a useful pharmacological approach. In 1997 the AMPA-analog, α-amino-5-tert-butyl-3-hydroxy-4-isoxazolyl)propionic acid (ATPA), was shown to be a highly selective ligand for the iGlu5 receptor subtype. Concurrently (4R)-methyl Glu (1a) was found to display preference for KA receptors over AMPA, NMDA, and the mGlu receptors ([BraunerOsborne et al., 1997], [Gu et al., 1995], [Sagot et al.,
2008] and [Zhou et al., 1997]), and in 2001 we reported that E-4-neopentylidene Glu (2f) (Bunch et al., 2001) is a highly selective iGlu5 receptor ligand (Table 1). In this paper we present the pharmacological investigation of a five-compound series of (4R)-alkyl Glu analogs (1b-e,g) which may be viewed as conformationally released analogs of 2b-e,g. Such a distinct – yet confined – change in physical chemical property is interesting as it allows for a detailed investigation of ligand flexibility as opposed to receptor subtype selectivity.

Table 1. Binding affinities of (4R)-alkyl Glu analogs (1a-e,g), E-4-alkylidene Glu analogs (2a-h), AMPA, ATPA, and KA at native iGlu receptors (rat brain synaptosomes) and at cloned rat homomeric iGlu5-7 receptor subtypes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>AMPA</th>
<th>KA</th>
<th>NMDA</th>
<th>iGlu5</th>
<th>iGlu6</th>
<th>iGlu7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>28,600</td>
<td>52</td>
<td>5000</td>
<td>0.7</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>1b</td>
<td>16,000</td>
<td>120</td>
<td>&gt;10,000</td>
<td>2.08</td>
<td>75.5</td>
<td>27.5</td>
</tr>
<tr>
<td>1c</td>
<td>78,000</td>
<td>70</td>
<td>&gt;75,000</td>
<td>11.31</td>
<td>458</td>
<td>141.6</td>
</tr>
<tr>
<td>1d</td>
<td>&gt;100,000</td>
<td>750</td>
<td>&gt;100,000</td>
<td>37.7</td>
<td>2270</td>
<td>363.5</td>
</tr>
<tr>
<td>1e</td>
<td>&gt;100,000</td>
<td>5000</td>
<td>&gt;100,000</td>
<td>63.5</td>
<td>4890</td>
<td>1956</td>
</tr>
<tr>
<td>1f</td>
<td>42,000</td>
<td>&gt;100,000</td>
<td>&gt;100,000</td>
<td>20.5</td>
<td>1000</td>
<td>6167</td>
</tr>
<tr>
<td>1g</td>
<td>150</td>
<td>230</td>
<td>1200</td>
<td>270</td>
<td>450</td>
<td>–</td>
</tr>
<tr>
<td>1h</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2200</td>
<td>2500</td>
<td>–</td>
</tr>
<tr>
<td>2a</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>61</td>
<td>18,000</td>
<td>–</td>
</tr>
<tr>
<td>2c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>13,000</td>
<td>&gt;40,000</td>
<td>–</td>
</tr>
<tr>
<td>2d</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>54</td>
<td>83,000</td>
<td>520</td>
</tr>
<tr>
<td>2f</td>
<td>2000</td>
<td>–</td>
<td>–</td>
<td>16.4</td>
<td>&gt;1000</td>
<td>2247</td>
</tr>
<tr>
<td>2g</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>224</td>
<td>36400</td>
<td>–</td>
</tr>
<tr>
<td>2h</td>
<td>6500</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>122</td>
<td>&gt;10000</td>
<td>27270</td>
</tr>
<tr>
<td>AMPA</td>
<td>460</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>2000</td>
<td>&gt;10000</td>
<td>48270</td>
</tr>
<tr>
<td>ATPA</td>
<td>1800</td>
<td>23000</td>
<td>&gt;10000</td>
<td>3</td>
<td>&gt;10000</td>
<td>2530</td>
</tr>
<tr>
<td>KA</td>
<td>4000</td>
<td>7</td>
<td>&gt;10000</td>
<td>76</td>
<td>13</td>
<td>33</td>
</tr>
</tbody>
</table>

2. Materials and methods

2.1. Binding affinities at native and homomeric ionotropic Glu receptors

Binding affinities for 1b-e,g at native AMPA, KA, NMDA receptors (rat synaptosomes) were determined according to the previously published experimental procedure (Hermit et al., 2004) using radioligands [3H]AMPA, [3H]KA (representing predominantly subtypes KA1,2), and [3H]CGP39653, respectively. Determination of binding affinities for 1b-e,g and 2f,h at cloned rat homomeric receptor subtypes iGlu5-7 were carried out following the procedure described earlier, using [3H]-SYM2081 as the radioligand (Sagot et al., 2008).
2.2. Molecular modelling

The modeling study was performed using the software package MOE (Molecular Operating Environment, v2004.03, Chemical Computing Group, 2004) using the build-in mmff94x forcefield and the GB/SA continuum solvent model. Compound 1g was submitted to a stochastic conformational search and with respect to its global minimum returned (∆G in kcal/mol), conformations above + 7 kcal/mol were discarded. The γ-carboxylate group was protonated prior to execution of the conformational search, as this gave a larger and thus more reliable number of output conformations. Superimpositions of ligands, were carried out using the built-in function in MOE, by fitting the ammonium group and the two carboxylate groups. The conformation of ATPA was adapted from X-ray structure when bound in the iGlu2 subunit (PDB: 1 nk).

3. Results

3.1. Binding affinities at native Glu receptor subtypes

In binding assays at native AMPA, KA and NMDA receptors, 1b shows preference for the KA receptors with decreasing affinity as the 4-substituent is enlarged in size and bulk (1a–e,g). Eventually, compound 1g displays very low affinity for any of the three iGlu receptor subgroups (42, > 100, > 100 µM, respectively, Table 1).

3.2. Binding affinities at cloned homomeric Glu receptor subtypes

At cloned homomeric iGlu5–7 receptor subtypes, Glu analog 1b displayed a 35- and 10-fold preference for iGlu5 over subtypes iGlu6,7, respectively. As the 4R-alkyl substituent is increased in length and bulkiness, this trend is strengthened (Table 1 and Fig. 1), and eventually Glu analog 1g is highly selective for iGlu5 receptor subtype (Kᵢ = 20.5 nM), displaying more than 5000-fold and 300-fold selectivity over iGlu6,7 receptor subtype, respectively (Table 2).

![Fig. 1. Binding affinities (pKᵢ [nM]) of (4R)-alkyl Glu analogs 1a–e,g (top) and E-4-alkylidene Glu analogs 2a–h (bottom) at cloned homomeric iGlu5–7 receptor subtypes.](image)

Table 2.
Receptor subtype selectivity calculated and normalized from data presented in Table 1 (iGlu$_5$ receptor subtype set to 1).

<table>
<thead>
<tr>
<th></th>
<th>AMPA</th>
<th>KA$_{1,2}$</th>
<th>NMDA</th>
<th>iGlu$_5$</th>
<th>iGlu$_6$</th>
<th>iGlu$_7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1g</td>
<td>2050</td>
<td>&gt; 5000</td>
<td>&gt; 5000</td>
<td>1</td>
<td>&gt; 5000</td>
<td>1</td>
</tr>
<tr>
<td>2f</td>
<td>80</td>
<td>&gt; 4200</td>
<td>&gt; 4200</td>
<td>1</td>
<td>&gt; 4200</td>
<td>90</td>
</tr>
<tr>
<td>2g</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>2830</td>
<td>–</td>
</tr>
<tr>
<td>ATPA</td>
<td>450</td>
<td>5750</td>
<td>&gt; 25,000</td>
<td>1</td>
<td>&gt; 25,000</td>
<td>2500</td>
</tr>
</tbody>
</table>

$^a$ Predominant KA receptor subtypes expressed in native rat synaptosomes.

4. Discussion

Despite the fact that Glu is a highly flexible molecule (Fig. 2), it has been shown in several X-ray crystallographic and medicinal chemistry studies, that Glu agonizes iGlu receptors in a well-defined conformation termed the *folded* conformation ([Bunch and Krosgaard-Larsen, 2009], [Bunch et al., 2003] and [Hogner et al., 2002]). On the other hand, when activating the mGlu receptors, Glu adopts an *extended* conformation ([Hayashi et al., 1992] and [Kozikowski et al., 1998]).

![Glu folded conformation](image1)

![Glu extended conformation](image2)

Fig. 2. Rotation of the C(2)–C(3) and C(3)–C(4) bonds allows Glu to adapt nine different staggered conformations. Of these, the two low-energy conformations are termed the *folded* and *extended* conformations. These two Glu conformations are also observed when Glu is crystallized with iGlu receptor subunits (e.g. the iGlu$_5$ subunit: PDB code: 1TXF) and mGlu$_1$ subunit (PDB code: 1EWK).

Recently we reported the synthesis and pharmacological evaluation of a series of (4$R$)-alkyl Glu analogs at EAAT$_{1-3}$ (Alaux et al., 2005). In extension from earlier findings, we showed that introduction of a large variety of longer and more bulky (4$R$)-alkyl substituents also endorses the *folded* conformation as the global low-energy conformation of Glu. In comparison, this low-energy conformational preference is also preferred for $E$-4-alkylidene Glu analogs 2a–h, AMPA, and ATPA (Fig. 3). Furthermore, a general feature for all of these Glu analogs, is the observed increase in preference for the iGlu$_5$ receptor subtype, as the alkyl group is extended in length and bulk (1a $\rightarrow$ 1g, 2a $\rightarrow$ 2f and AMPA $\rightarrow$ ATPA; Table 1). This observation may be explained by comparing the size of the iGlu receptor binding pockets: For subunit iGlu$_5$, it has been estimated to be approximately 20% larger as compared to subunit iGlu$_6$ and approximately 50% larger, when compared to subunit iGlu$_2$ (305 Å, 255 Å, and 218 Å, respectively) (Mayer, 2005).

![Superimposition of low-energy conformations](image3)

Fig. 3. Superimposition of low-energy conformations of 1g ($\Delta \Delta G = 0$ to $+1$ kcal/mol) (type code/green) and ATPA (purple). The conformation of 1g shown in green resembles the conformation of ATPA best.
(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(4R)-Alkyl Glu analogs 1a–e,g are conformationally released structural analogs of 4-alkylidenes 2a–h. This confined modification of physical chemical property may allow for a detailed investigation of ligand flexibility opposed to receptor subtype selectivity. Comparing the binding affinity profiles of series 1 and 2 at iGlu_6,7 receptor subtypes reveals that both series display increasing selectivity as well as binding affinity for iGlu_5, as the 4-substituents is extended in length and bulk. However, from this resemblance two divergences are noted: Firstly, 1g vs. 2g displays a 10-fold difference in binding affinity at the iGlu_5 receptor subtype (K_i = 20.5 and 224 nM, respectively). This finding is intriguing and raises the question as to what origin this observation has. One explanation could be that the more flexible ligand, 1g, may allow for a more tight domain closure, favoring both ligand–receptor and receptor–receptor interactions. A different explanation could be enhanced hydrophobic interactions with the receptor protein. Secondly, 2d stands out from the trend, displaying significantly lower binding affinity to the iGlu_5 receptor subtype as compared to 2e and 2e, as well as 1c–e. However, this is explained on the basis that 2d is structurally quite distinct from preceding analogs 2a–c and subsequent analogs 2e–f, as it has a methyl group in the Z-1' position as opposed to hydrogen.

In conclusion, Glu analogs 1b–e,g are a conformationally released structural design of 2a–h and ATPA. Most notably, 1g displays high affinity for the KA receptor subtype iGlu_5 with a high degree of selectivity over receptor subtypes iGlu_6,7 (fold ratio: > 5000, 300, respectively), KA subtypes KA_1/KA_2 (fold ratio: > 5000, determined by KA binding at native rat synaptosomes), AMPA receptors (2050 fold, determined by AMPA binding at native rat synaptosomes), and NMDA receptors (> 5000 fold, determined by CGP39653 binding at native rat synaptosomes), making 1g a valuable pharmacological tool. Furthermore, 1g displays an intriguing 10-fold higher binding affinity at iGlu_5 receptor subtype than its corresponding E-4-alkylidene isomer 2g. This observation encourages an expansion of the (4R)-alkyl Glu series by the synthesis of corresponding saturated analogs of 2f,h, and a following investigation whether such difference in binding affinity at iGlu_5 receptor subtype is a generally observable fact and ultimately if it translates into functional differences.

Acknowledgements
We would like to thank the Carlsberg Foundation, the Lundbeck Foundation, and the Danish Research Council for financial support.

References


