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Design of new analogues of glutamic acid with a conformationally restricted structure

Paola Conti, Marco De Amici, Carlo De Micheli *

Istituto di Chimica Farmaceutica e Tossicologica, Viale Abruzzi 42, 20131 Milan, Italy

Abstract

Regioisomeric 3-carboxyisoxazolinyl prolines (CIP-A and CIP-B) and 3-hydroxyisoxazolinyl prolines [(±)-**8** and (±)-**9**] were synthesized and assayed for glutamate receptor activity. CIP-A [(±)-**6**] showed a convulsant activity evaluated in vivo on DBA/2 mice, higher than AMPA and similar to kainic acid. The eutomer of CIP-A [CIP-AS, (−)-**6**], obtained from (*S*)-3,4-didehydroproline, evidenced common stereochemical requirements with AMPA and kainic acid. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Glutamate receptors; Convulsant activity; Isoxazolinylprolines; AMPA agonists; Kainic acid agonists

The amino acids glutamate [(*S*)-Glu, **1**] and aspartate [(*S*)-Asp, **2**], present in the mammalian CNS, are the major excitatory neurotransmitters [1]. These excitatory amino acids play an important role in many physiological functions including learning, memory and other forms of synaptic plasticity [2–4]. Several classes of glutamate receptors, widely distributed throughout the CNS, have been identified, cloned and characterized [5–8]. Three classes belong to the family of ionotropic receptors (iGluRs) [5,6] and were classified according to activation by specific agonists, *N*-methyl-D-aspartate (NMDA, **3**), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA, **4**) and kainic acid (KAIN, **5**) (Scheme 1).

Molecular biology has since resulted in the cloning and functional expression of a variety of NMDA, AMPA and KAIN receptor subunits. As an example, to date four AMPA receptor subunits (GluR1-4) and five KAIN receptor subunits (GluR5-7, KA1 and KA2) have been cloned (rat and human) [7]. Human AMPA receptor subunits, when expressed homomerically in host cells, can form functional ion channels which can be activated by both AMPA and KAIN. Of the five KAIN receptor subunits, GluR5, GluR6 and GluR7 form functional ion channels on homomeric expression. These channels are activated by KAIN while AMPA has very little activity. On the other hand KA-1 and KA-2, the high affinity KAIN subunits, do not form homomeric channels but their co-expression with GluR5, GluR6 and GluR7, the low affinity KAIN receptor subunits, yields functional channels.

Glutamic acid also activates an heterogeneous family of metabotropic receptors (mGluRs) [7,8] which regulate, via a G-protein, the activity of membrane enzymes such as phospholipase C (PLC) or adenylate cyclase (AC). Molecular cloning has revealed the existence of at least eight subtypes of mGluRs.

Overactivation of glutamate receptors has been implicated in neuronal degeneration and loss in acute conditions such as head injury, stroke and epileptic seizures, as well as in chronic neurodegenerative diseases including Alzheimer's, Huntington's and Parkinson's diseases [9].

A prerequisite for the determination of the physiological role and pharmacological importance of the iGluRs and mGluRs subtypes is the availability of highly selective agonists and antagonists. A number of agonists and antagonists, acting selectively at AMPA or KAIN receptors, have been reported [10]. Nevertheless there is still a demand for ligands capable to discriminate their receptor subtypes.

We enter this field with the aim to design new analogues of glutamic acid as structural hybrids of AMPA and KAIN, which could be subsequently modified to increase the selectivity for one class of iGluRs. Regioisomeric 3-carboxyisoxazolinyl prolines

* Corresponding author.

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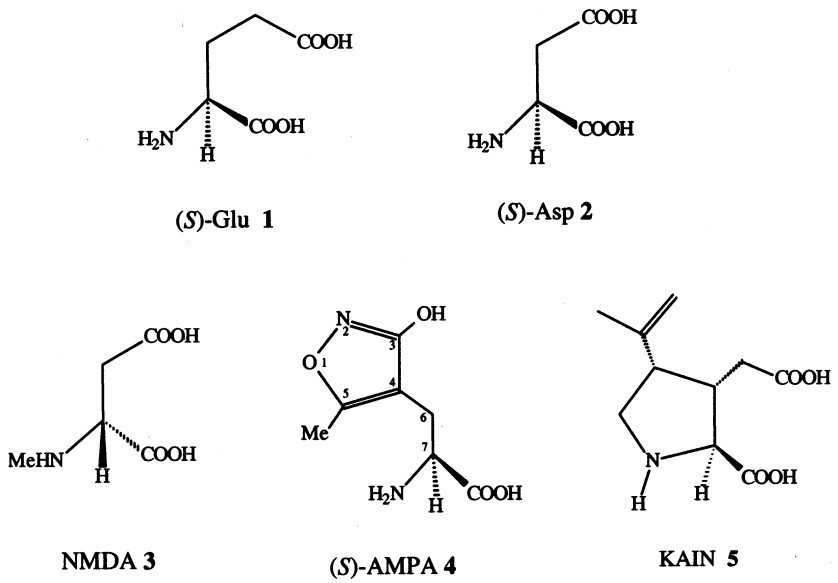
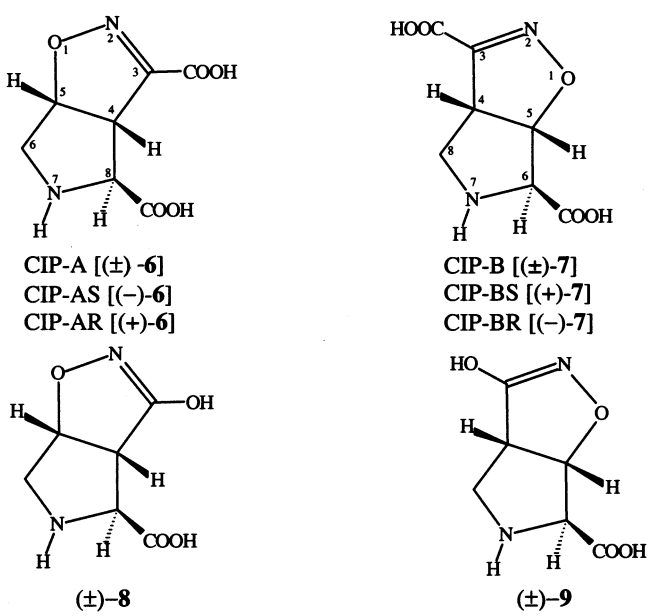


Scheme 1.

[CIP-A, (±)-**6** and CIP-B, (±)-**7**] and 3-hydroxyisoxazolinyl prolines [(±)-**8** and (±)-**9**] (Scheme 2) were prepared and assayed for glutamate receptor activity. The key step in the synthesis of target compounds (±)-**6**, (±)-**7**, (±)-**8**, and (±)-**9** is represented by the 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide or bromoformonitrile oxide to the suitably protected racemic 3,4-didehydroproline. The pericyclic reaction gave three out of the four possible stereiosomers, which were separated by column chromatography and subsequently transformed into final derivatives through a sequence of conventional reactions. The same reaction sequence was also carried out on (*S*)- and (*R*)-3,4-didehydroproline to yield the two couples of diastereomers CIP-AS/CIP-BS [(−)-**6**/(+)-**7**] and CIP-AR/CIP-BR [(+)-**6**/(−)-**7**], respectively.



Scheme 2.

The activity of amino acids **6**, **7**, **8**, and **9** at AMPA and KAIN receptors was assayed in vitro by means of receptor binding techniques, second messenger assays, and the rat cortical wedge preparation. The convulsant properties of all the compounds were evaluated in vivo on DBA/2 mice after icv injection. CIP-A and CIP-AS are provided with a remarkable convulsant activity, at variance with the results collected in in vitro tests where they showed a moderate binding affinity at both AMPA and KAIN receptors. The convulsant activity of CIP-A, measured as tonic and clonic seizures, was 18–65 times higher than that displayed by AMPA and only 2–5 times lower than that shown by KAIN. CIP-AS [(−)-**6**] turned out to be the eutomer with a convulsant potency similar to that displayed by KAIN and 90–117 times higher than that shown by AMPA. The convulsant potency of CIP-B and CIP-BS is two orders of magnitude lower than those shown by their stereoisomers CIP-A and CIP-AS. Derivatives (+)-**6**, (−)-**7**, (±)-**8**, and (±)-**9** are almost inactive. CIP-AS [(−)-**6**] is also quite active by ip administration since it is able to induce seizures in mice at doses as low as 108 μmol kg^{-1} showing a potency comparable with that of KAIN (89 μmol kg^{-1}) and higher than that of AMPA (143 μmol kg^{-1}).

Since AMPA and its analogues are characterized by the presence of a 3-hydroxyisoxazole nucleus, we can deduce that such a structural feature can lead to compounds characterized by a selective affinity for the AMPA receptor complex. The replacement of the 3-hydroxyisoxazole nucleus of AMPA-selective ligands with the 3-carboxyisoxazolinyl moiety gives compounds, e.g.

CIP-A and CIP-AS, in which the spatial arrangement of the pharmacophoric groups is suitable for an additional interaction with the KAIN receptor subsites. The affinity of CIP-A and its eutomer CIP-AS for the KAIN receptor complex is noteworthy. Since the spatial arrangement of the groups around the chiral centers of CIP-AS is identical to that of the eutomer of AMPA [(*S*)-AMPA] and of natural KAIN, we deduced that the stereochemical features of CIP-AS are suitable for a productive interaction with both AMPA and KAIN receptors. On the contrary, the replacement the 3-hydroxyisoxazole nucleus of AMPA with the 3-hydroxy-Δ^2-isoxazolinyl moiety, e.g. (±)-**8**, and (±)-**9**, almost abolishes the affinity for both AMPA and KAIN receptors even in the case of (±)-**9**, whose through-bond connection of the pharmacophoric groups matches that of glutamic acid. Two conceivable explanations can be proposed to account for the lack of activity of (±)-**9**. The first one is based on a different spatial arrangement of the pharmacophoric groups between (±)-**9** and the reference compounds AMPA and KAIN. A comparative conformational profile of (±)-**9** with that of model compounds was undertaken. The second hypothesis takes into account the acidity of the ω-hydroxyl group. The measurement of the pK_{a_2} of (±)-**9** showed a value similar to that reported for AMPA. As a consequence, the acidity of the 3-hydroxyl group of (±)-**9** can not be taken as the cause of its inactivity at AMPA and KAIN receptors. Modeling studies carried out with the aid of theoretical calculations indicate that the lack of activity of stereoisomers (±)-**8**, and (±)-**9** at iGluRs can not be attributed to the inability of their 3-hydroxy-Δ^2-isoxazoline moiety to behave as an efficient bioisoster of the ω-carboxylate group of glutamic acid but, more likely, to an inappropriate spatial arrangement of their pharmacophoric groups. On the other hand, the capability of CIP-A and, especially, of CIP-AS to activate both the AMPA and KAIN receptors has to be ascribed to the presence in their structure of two conformations which are equally populated and mimic an active conformation of AMPA and KAIN, respectively.

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