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Conformationally Constrained Analogues of L-Glutamate as Subtype-Selective Modulators of Metabotropic Glutamate Receptors

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L-Glutamate, a major neurotransmitter in excitatory synaptic pathways of the mammalian central nervous system (CNS)(*1*), plays an important role in many integrative brain functions (*1–4*). Glutamate receptors have been classified into two distinctive groups termed ionotropic and metabotropic receptors (*1–4*). The ionotropic receptors (iGluRs) consist of *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors that contain glutamate-gated, cation-specific ion channels. The metabotropic glutamate receptors (mGluRs), however, are different both functionally and pharmacologically from ionotropic receptors. They are coupled to G-proteins that mediate a variety of transduction mechanisms(*5*). In recent years we have witnessed a growing interest in the field of mGluRs, due to the intriguing therapeutic opportunities offered by the modulation of its members(*6*). To date eight cloned mGluRs have been characterized and classified into three subgroups according to their sequence homology, signal transduction mechanism, and agonist selectivity (*7–9*). The group I mGluRs include mGluR1 and mGluR5, which are potently activated by quisqualate resulting in an increase in phosphoinositide hydrolysis. In contrast, group II including mGluR2 and mGluR3 and group III that contains mGluR4, mGluR6, mGluR7, and mGluR8 are negatively linked to adenylyl cyclase. However, they can be distinguished by their marked agonist selectivity. The former effectively interacts with (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I), whereas the latter potently interacts with (*S*)-2-amino-4-phosphonobutyric acid (L-AP4). Preliminary studies have revealed that the different subtypes may contribute differently to the regulation of synaptic transmission, as well as in the etiology of neurological disorders (*7–10*). These disorders include epilepsy, focal and global ischemia, pain, and neurodegenerative diseases. In order to better characterize the roles of mGluRs in physiological processes, there is an important need to develop novel, high affinity ligands which are family and subtype specific. Many advances have been made in the identification of useful ligands with subtype selectivity in the

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past 5 years. From a structural viewpoint, these new ligands are actually analogues of L-glutamate. In the course of designing mGluR ligands, two major modification methods were often used, one was bioisosterism and the other was incorporation of conformational constraints. For space reasons, this review will focus only on the discussion of the structural features of these ligands as agonists or antagonists. For discussions of the neurophysiological roles of specific mGluR subtypes, refer to other excellent reviews (*1–9*).

According to their structural difference, mGluR ligands can be divided into four major classes, namely analogues of ACPD, analogues of CCG, analogues of phenylglycine, and analogues of L-AP4. Other ligands with unrelated structures are discussed in the fifth section.

1. ANALOGUES OF ACPD

(1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) was originally prepared as a conformationally constrained analog of glutamate in 1988 (*11*). One year later it was found to be a selective agonist for mGluRs over iGluRs (*12*). The selectivity of this compound for mGluRs was believed to be at least in part due to the conformational rigidity that is imparted by the cyclopentane ring. Since this compound was first identified as a selective agonist for mGluRs, it has played a crucial role in seeking the functions of *in situ* mGluRs (*1–9*). Further studies showed that (1*S*,3*R*)-ACPD did not have marked subtype selectivity for different mGluRs; however, its 3-epimer, (1*S*,3*S*)-ACPD shows negligible activity at group I receptors and is good agonist of group II mGluRs (*13,14*). Based on these observations, several groups have carried out a modification to the structure of (1*S*,3*R*)-ACPD in order to find more potent or selective ligands. Lilly's researchers reported that an aza-substituted analogue, (2*R*,4*R*)-APDC, had about two times greater potency (EC_{50} = 3.5 μM at human mGluR2) of activating group II mGluRs than (1*S*,3*R*)-ACPD but had little effect on group I mGluRs (*15,16*). Conformation studies showed that this compound preferentially adopted a fully extended glutamate conformation in the vacuum phase, which gave an additional support for the hypothesis that L-glutamate interacts with group II mGluR protein in a fully extended form (*16*). Benzylation of (2*R*,4*R*)-APDC led to 1-benzyl-APDC, which was found to have weak antagonist activity for group I and II mGluRs (IC_{50} = 600 and 200 μM at mGluR5 and mGluR2, respectively) but to activate mGluR6 selectively (EC_{50} = 20 μM) (Fig. 1) (*17*).

By introducing an additional carboxylic group at the 4-position of ACPD, Azerad and co-workers synthesized four diastereoisomers, ACPT-I, ACPT-II, (+)-ACPT-III, and (−)-ACPT-III. Biological evaluation showed that ACPT-II was a nonselective and weak antagonist for group I, II, III mGluRs, while (−)-(3*R*,4*R*)-ACPT-III was a competitive antagonist showing poor but significant affinity for group III receptor mGluR4a (K_B = 220 μM). Both ACPT-I and (+)-(3*S*,4*S*)-ACPT-III were found as potent and selective agonists for mGluR4a with EC_{50} values of 7.2 and 8.8 μM, respectively (*18*). These results implied that if the additional carboxylic group was trans to the 1-amino group the generated analogues of (1*S*,3*S*)-ACPD (ACPT-I) or (1*S*,3*R*)-ACPD ((+)-(3*S*,4*S*)-ACPT-III) would keep the agonist activity but change selectivity to group III mGluRs, while the additional carboxylic group was cis to the 1-amino group, the generated analogues would convert agonist to antagonist activity.

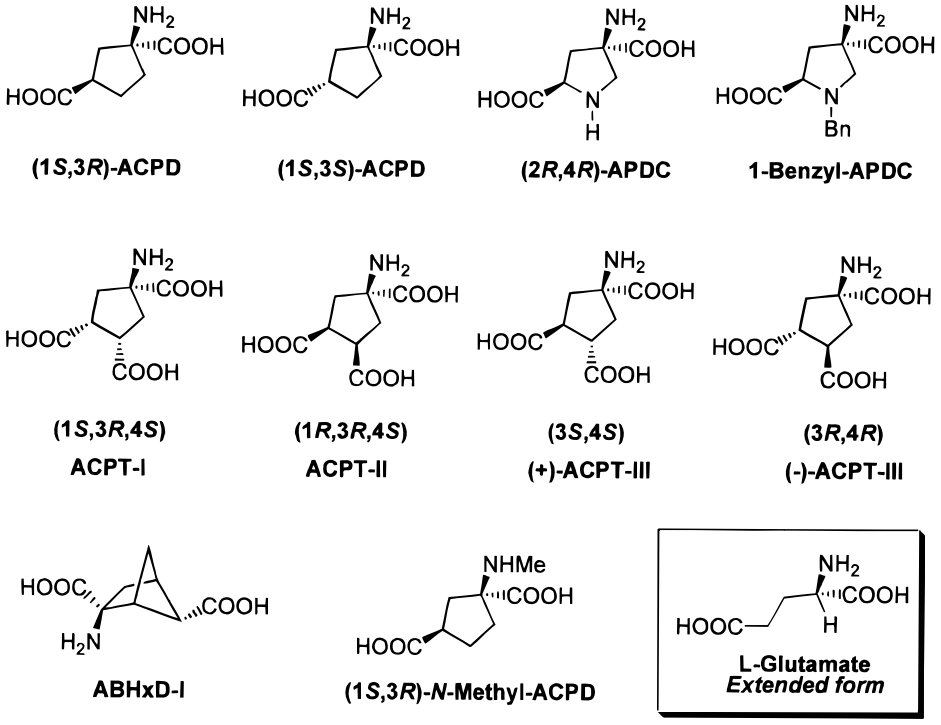

FIG. 1. Structures of analogues of ACPD.

Recently, Kozikowski's group found that if a methylene group was used to connect the 2- and 4-positions of (1*S*,3*S*)-ACPD, the resultant rigid analogue (1*S*,2*S*,4*S*,5*S*)-2-aminobicyclo[2.1.1]hexane-2,5-dicarboxylic acid (ABHxD-I) was a selective agonist for the mGluR family of receptors, acting with approximately the same selectivity and potency as L-glutamate (*19*). Computer modeling studies suggested that this compound might adopt only the fully extended conformation of L-glutamate. These results implied that the active conformation of (1*S*,3*R*)-ACPD or L-glutamate at each of mGluR subtypes could be that which best mimics an extended conformation.

By using L-malic acid as starting material, a stereoselective synthesis of (1*S*,3*R*)-ACPD was achieved (*20*), and (1*S*,3*R*)-*N*-methyl-ACPD was synthesized following this protocol. This compound had no activity when it was tested as either agonist or antagonist for mGluRs (*21*). Thus, it seemed that *N*-alkylation of (1*S*,3*R*)-ACPD would lead to loss of the affinity to mGluRs.

2. ANALOGUES OF CCG

In order to have better knowledge of the conformational requirements of L-glutamate for activating each glutamate receptor subtype, Ohfune and co-workers synthesized four diastereomers of L-2-(carboxycyclopropyl)glycine (L-CCG-I–IV), conformationally restricted analogues in which a cyclopropyl group fixes the glutamate chain in

either an extended or a folded form (*22*). Among these compounds, (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine could selectively activate the mGluRs with a similar potency as L-glutamate, while L-CCG-IV was found as a selective and potent agonist for NMDA receptor. Further studies demonstrated that L-CCG-I had the subtype selectivity for group II mGluRs (*3*). These observations led them to conclude that NMDA receptors are stimulated by the folded form of L-glutamate, while mGluRs, at least group II mGluRs, are activated by the extended form of L-glutamate. As noted before, this hypothesis was further supported by the results of (2*R*,4*R*)-APDC (*15*,*16*).

Based on the structure of L-CCG-I (Fig. 2), several groups have tried to introduce an additional substituted group at the 3′-position to obtain new ligands. These efforts have led to the discovery of some new ligands with higher potency and better selectivity or different actions for mGluRs in recent years. These results also gave some insight into how L-CCG-I interacts with group II mGluR proteins. It was found that the substituted groups and their special orientation could dramatically influence the activity to mGluRs. For examples, (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl) glycine (L-DCG-IV), an analogue of L-CCG-I by adding a third carboxylic group at the 3′-position which is geometrically trans to the 2′-carboxylic group (*23*), was about 10 times more potent than L-CCG-I for activating group II mGluRs and also possessed better subtype selectivity for mGluRs (*24*). However, it should be noted that L-DCG-IV must be used with caution as a tool for the investigation of mGluRs because this compound was also found to be a potent agonist for NMDA receptor (*25*). Similarly, both *trans*- and *cis*-MCG-I, two analogues with an additional methoxymethyl group cis or trans to the 2′-carboxylic group on the cyclopropane ring, were found to be more selective agonists than L-CCG-I. The potency of *trans*-MCG-I was 2 times that of *cis*-MCG-I and almost the same as that of L-CCG-I (*26*,*27*). Replacement of the two protons at the 3′-position with two fluorine atoms led to (2*S*,1′*S*,2′*S*)-2-(2-carboxy)-3,3-difluorocyclopropyl)glycine (L-F_2CCG-I), which was found to preferentially depress the monosynaptic component of the spinal reflex approximately 3 times more effectively than L-CCG-I (*28*). However, if a bulky group such as a phenyl group was introduced at the 3′-position cis to the 2′-carboxylic group, the resultant

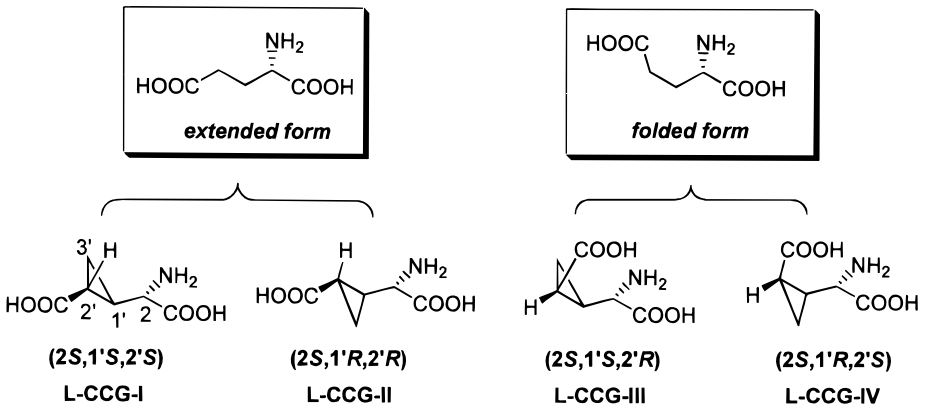

FIG. 2. Structures of L-CCG-I–IV.

analogue PCCG-12 lost its activity for the mGluRs, while analogue PCCG-4 with a 3′-phenyl group trans to 2′-carboxylic group was found to display selective antagonist activity for group II receptor mGluR2 (IC_{50} = 8 μM) (*29*).

Incorporation of a substituent on the amino acid carbon of L-CCG-I (Fig. 3) was also found to be a strategy for converting agonist to antagonist activity. This phenomenon was first reported by Watkins's laboratory (*30*). They found that (2*S*,1′*S*,2′*S*)-2-methyl-(2′-carboxycyclopropyl)glycine (MCCG), a 2-methyl analogue of L-CCG-I, was a selective antagonist for group II mGluRs with moderate potency. At this point, Lilly's chemists have undertaken intensive investigation (*31*,*32*). By varying the lipophilic side chains, they synthesized dozens of analogues of L-CCG-I. Based on structure-activity studies they obtained the following conclusion:

(1) Alkyl substitution, both normal and terminally branched, all lowered affinity relative to the unsubstituted compound. For example, the most potent compound among the group of alkyl-substituted compounds, isopropyl analogue **1a** (R = *i*-Pr, Fig. 4) was about 2-fold less active than L-CCG-I.

(2) In examining several phenylalkyl-substituted compounds **2**, only phenylethyl analogue **2a** (n = 1) showed slightly higher potency than L-CCG-I. Other analogues with a long chain (n > 1) or short chain (n = 0) all displayed lower affinity than L-CCG-I.

(3) Putting additional substituents on the aromatic ring of **2a** could also alter the affinity. Meta substitution of a variety of functional groups consistently provided a significant increase in affinity for mGluRs, ranging from 1.4- to 14-fold over unsubstituted compounds. Both electron-donating and electron-withdrawing substituents gave increases in affinity at this position. However, ortho substitution was either more or less effective or deleterious to affinity while para substitution was in general deleterious.

(4) Among the tested diphenylalkyl-substituted compounds **3**, diphenylethyl-substituted compound **3a** (n = 0) showed higher affinity relative to L-CCG-I. Long chain analogues (n > 0) all showed lower affinity than L-CCG-I.

(5) Greater increase in potency could be achieved when the two phenyl rings of **3a** were bound together by an oxygen or sulfur atom.

These studies have led to the discovery of several highly potent and selective

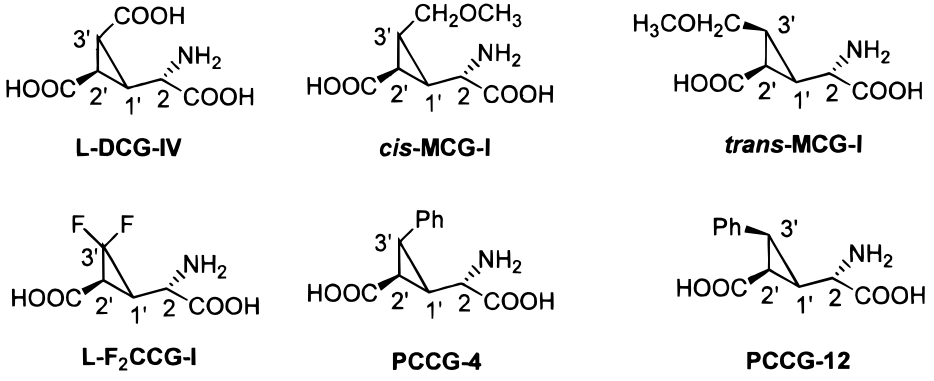

FIG. 3. Stuctures of 3′-substituted analogues of L-CCG-I.

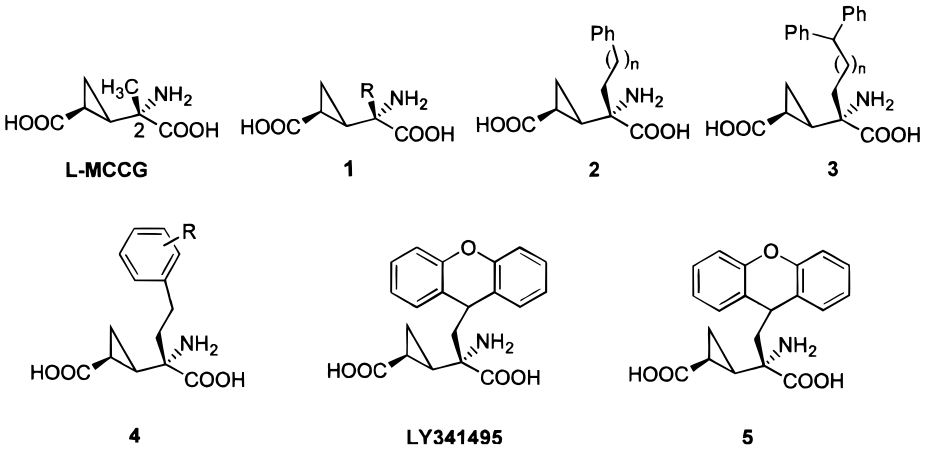

FIG. 4. Structures of 2-substituted analogues of L-CCG-I.

antagonists for group II mGluRs represented by LY341495, the most potent and selective antagonist for group II mGluRs reported to date. The biological data of some antagonists are summarized in Table 1. In addition, some of these compounds also showed *in vivo* activity. For example, systemic administration of LY341495 has shown the ability to protect mice from limbic seizures produced by the mGluR agonist 3,5-dihydroxyphenylglycine with an ED_{50} = 31 mg/kg. It was also found that a low dose of LY341495 could block the anxiolytic effects of an mGluR agonist in the elevated plus maze in mice. However, the oral bioavailability of this compound was low (~5%), which would limit its further application as a therapeutic agent.

By computer-assisted comparison of the low-energy conformers of (1*S*,3*R*)-ACPD, L-CCG-I and (2*R*,4*R*)-APDC, Lilly's chemists realized that a fully extended glutamate backbone was required for optimal group II mGluR protein-ligand interactions. To test this hypothesis, they designed and synthesized a bicyclic amino acid, LY354740 in which the glutamic acid skeleton was incorporated into a fused bicyclo[3.1.0]hexane nucleus (Fig. 5). The hypothesis was supported by the fact that LY354740 could interact with high affinity (EC_{50} = 5.5 nM at human mGluR2) and specificity (no action for group I mGluRs at concentrations up to 100 μM) at group II mGluRs. More importantly, LY354740 also showed oral activity in NIH Swiss mice both in the elevated plus maze model of anxiety (ED_{50} = 0.5 mg/kg) and in the ACPD-induced limbic seizure model (ED_{50} = 45.6 mg/kg), and may be a valuable tool for

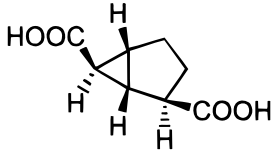

FIG. 5. Structure of LY354740.

TABLE 1

Affinities and Functional Antagonist Activity of 2-substituted analogues of L-CCG-I for Group II mGluRs(*29, 30*)

Amino acid	ACPD-sensitive [^{3}H]glutamate binding IC_{50} (μM)	Antagonist activity at human mGluR2 IC_{50} (μM)	Antagonist activity at human mGluR3 IC_{50} (μM)
L-CCG-I	0.47	Agonist	Agonist
3a (n = 0)	0.24 ± 0.08	4.36	0.28
4a (R = 4-F)	0.022 ± 0.002	0.18	0.21
4b (R = 3-COOH)	0.077 ± 0.024	1.8	0.08
LY341495	0.0029 ± 0.0006	0.023 ± 0.004	0.010 ± 0.008
5	0.019 ± 0.003	—	—

studying the effects of group II mGluR agonists in human (*10,33*). Although this result clearly showed that the fully extended conformation of L-glutamate was necessary for optimal group II mGluR protein-ligand interactions, it should be noted that the Kozikowski's result suggested that this extended conformation could be recognized by the majority of the mGluR subtypes (*19*). Thus, the subtype selectivity expressed by LY354740 might result from the subtle conformation difference.

Taking these studies together, we could summarize a putative pharmacophore for L-CCG-I and related compounds based on Pallicciari's hypothesis (*29*). As seen in Fig. 6, a fully extended form between two polar centers of these compounds is needed for interacting with receptor sites. Introducing a hydrogen-binding acceptor group at the 3′-position would lead to enhancement of agonist potency or selectivity in the orientations of either cis or trans to the 2′-carboxylic acid group, while adding a bulky group at the 3′-position trans to the 2′-carboxylic group would result in antagonist activity. Furthermore, replacement of the 2-hydrogen with a bulky group, in most cases, would switch its agonist to antagonist activity.

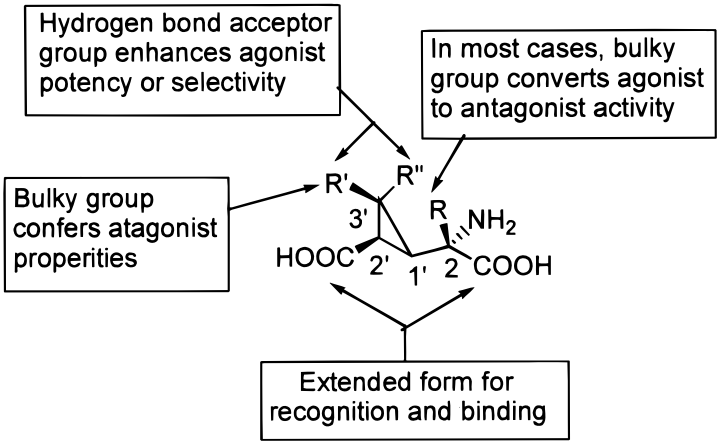

FIG. 6. Possible pharmacophores for L-CCG-I analogues.