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# New analogues of ACPD with selective activity for group II metabotropic glutamate receptors

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**Abstract**

In this study we have determined the pharmacology of a series of 1-aminocyclopentane-1,3-dicarboxylic acid (1,3-ACPD) analogues at cloned metabotropic glutamic acid (mGlu) receptors. The new analogues comprise the four possible stereoisomers of 1-amino-1-carboxycyclopentane-3-acetic acid (1,3-homo-ACPD) and the racemic mixture of (1*RS*,2*RS*)-1-amino-1-carboxycyclopentane-2-acetic acid (1*RS*,2*RS*-homo-ACPD). (1*RS*,2*RS*)-Homo-ACPD was shown to be a competitive $mGlu_2$ receptor antagonist with a $K_B$ of 391 μM. (1*S*,3*R*)-Homo-ACPD and (1*R*,3*R*)-homo-ACPD were both shown to be $mGlu_2$ receptor agonists with $EC_{50}$ values of 122 and 105 μM, respectively. Compared to (*S*)-Glu both compounds displayed partial agonism with intrinsic activities of 79% and 47%, respectively. (1*S*,3*S*)-Homo-ACPD was also found to be a partial $mGlu_2$ receptor agonist with an intrinsic activity of 27% compared to (*S*)-Glu. None of the compounds tested showed any activity at $mGlu_{1\alpha}$ or $mGlu_{4a}$ receptors. These homo-ACPD's show a higher degree of subtype selectivity than the parent compound (1*SR*,3*RS*)-ACPD. In addition none of the compounds demonstrated any activity at ionotropic Glu receptors. © 1997 Elsevier Science B.V.

*Keywords:* Metabotropic glutamic acid receptor; (1*SR*,3*RS*)-ACPD ((1*SR*,3*RS*)-1-Aminocyclopentane-1,3-dicarboxylic acid); (1*RS*,2*RS*)-homo-ACPD ((1*RS*,2*RS*)-1-amino-1-carboxycyclopentane-2-acetic acid); (1,3)-homo-ACPD (1-Amino-1-carboxycyclopentane-3-acetic acid); (Partial agonist); $mGlu_2$ receptor selectivity

## 1. Introduction

(*S*)-Glutamic acid ((*S*)-Glu) is the major excitatory amino acid transmitter in the central nervous system and plays an important role in many neuronal processes such as neural plasticity, memory and neurotoxicity (Choi and Rothman, 1990; Watkins et al., 1990; Bliss and Collingridge, 1993; Nakanishi and Masu, 1994). (*S*)-Glu mediates these effects through both ion channel receptors and G-protein coupled receptors (Nakanishi and Masu, 1994). The former class of receptors comprises the NMDA, AMPA and kainic acid subgroups of receptors which are all heterogeneous. So far eight different clones of the G-protein coupled metabotropic Glu (mGlu) receptors have been identified (Knöpfel et al., 1995). Based on pharmacology, sequence homology and the signal transduction pathway the mGlu receptors have been subclassified into three groups. The $mGlu_1$ and $mGlu_5$ receptors form group I, which are coupled to hydrolysis of phosphatidylinositol (PI) and are selectively activated by (*RS*)-3,5-dihydroxyphenylglycine (Brabet et al., 1995). Group II comprises $mGlu_2$ and $mGlu_3$ receptors which are negatively coupled to adenylate cyclase and are selectively activated by (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (Hayashi et al., 1993). Finally, the $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors belong to group III, which are also negatively coupled to adenylate cyclase and are selectively activated by (*S*)-2-amino-4-phosphonobutyric acid (L-AP4) (Knöpfel et al., 1995).

(1*SR*,3*RS*)-1-Aminocyclopentane-1,3-dicarboxylic acid (previously named *trans*-ACPD), of which (1*S*,3*R*)-ACPD is the active enantiomer, has been an important pharmacological tool due to its selective activation of mGlu recep-

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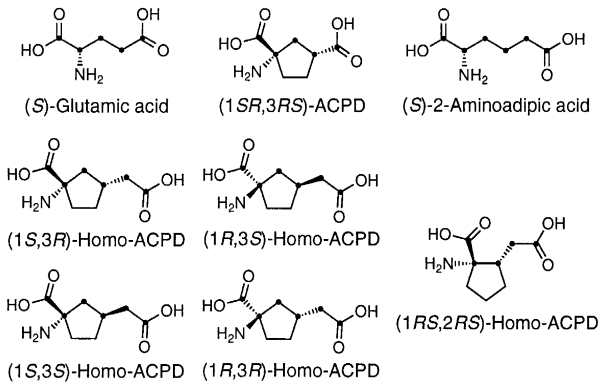


Fig. 1. Structures of (*S*)-Glu, (1*SR*,3*RS*)-ACPD, (*S*)-2-aminoadipic acid and the new ACPD analogues.

tors, although it rather non-selectively activates both group I and group II receptors (Knöpfel et al., 1995; Schoepp et al., 1995). In order to investigate the structure–activity relationship of ACPD analogues we have designed a series of compounds in which the carboxylic acid side-chain of ACPD has been extended to give the homologous compounds with an acetic acid side-chain. All four stereoisomers of 1-amino-1-carboxycyclopentane-3-acetic acid (1,3-homo-ACPD) as well as one regioisomer (1*SR*,3*RS*)-1-amino-1-carboxycyclopentane-2-acetic acid ((1*SR*,3*RS*)-homo-ACPD) have been investigated (see Fig. 1). In this study we report the pharmacology of these compounds at representative mGlu receptor subtypes expressed in Chinese hamster ovary (CHO) cells.

## 2. Materials and methods

### 2.1. Cell culture

The Chinese hamster ovary cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$ receptors have been described previously (Aramori and Nakanishi, 1992; Tanabe et al., 1992, 1993). They were maintained at 37°C in a humidified 5% $CO_2$ incubator in Dulbecco's Modified Eagle Medium (DMEM) containing a reduced concentration of (*S*)-glutamine (2 mM) and were supplemented with 1% proline, penicillin (100 U/ml), streptomycin (100 mg/ml) and 10% dialyzed fetal calf serum (all GIBCO, Paisley). Two days before assay $1.8 \times 10^6$ cells were divided into the wells of 24 well plates.

### 2.2. Second messenger assays

PI hydrolysis was measured as described previously (Hayashi et al., 1992, 1994). Briefly, the cells were labeled with [$^3$H]inositol (2 μCi/ml) 24 h prior to the assay. For agonist assays, the cells were incubated with ligand dissolved in phosphate-buffered saline (PBS)-LiCl for 20 min, and agonist activity was determined by measurement of the level of $^3$H-labeled mono-, bis- and tris-inositol phosphates by ion-exchange chromatography. For antagonist assays, the cells were preincubated with the ligand dissolved in PBS-LiCl for 20 min prior to incubation with ligand and 10 μM (*S*)-Glu for 20 min. The antagonist activity was then determined as the inhibitory effect of the (*S*)-Glu mediated response. The assay of cyclic AMP formation was performed as described previously (Hayashi et al., 1992, 1994). Briefly, the cells were incubated for 10 min in PBS containing the ligand and 10 μM forskolin and 1 mM 3-isobutyl-1-methylxanthine (IBMX) (both Sigma, St. Louis, MO, USA). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic AMP formation. For antagonist assay, the cells were preincubated with ligand dissolved in PBS containing 1 mM IBMX for 20 min prior to a 10 min incubation in PBS containing the ligand, 20 μM ($mGlu_2$) or 50 μM ($mGlu_{4a}$) (*S*)-Glu, 10 μM forskolin and 1 mM IBMX.

### 2.3. Data analysis

All experiments were performed in triplicate and the results are given as mean ± S.E.M. of at least three independent experiments. Antagonist potency was calculated from the Gaddum equation $K_B = [B]/(DR - 1)$ (Lazareno and Birdsall, 1993), where the dose-ratio (DR) is the ratio of the $EC_{50}$ values of (*S*)-Glu in the presence and in the absence of a fixed antagonist concentration, [*B*]. The synthesis of the new ACPD analogues will appear in a later publication, but the compounds can be obtained from Precision Biochemicals (Vancouver, Canada).

## 3. Results

The structures of the homo-ACPD's tested in this study are shown in Fig. 1. These ligands were initially tested in 1 mM concentration at CHO cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$ receptors representing group I, II and III, respectively. As seen in Fig. 2 none of the homo-ACPD's had any effect at $mGlu_{1\alpha}$ (panel A) or $mGlu_{4a}$ (panel C) when added alone or in combination which a submaximal concentration of (*S*)-Glu indicating that these ligands are inactive as agonists and antagonists at these receptors. (1*RS*,2*RS*)-Homo-ACPD had no activity at $mGlu_2$ when added alone, but could partially antagonize the (*S*)-Glu induced response indicating antagonism. Consistent with this result, 1 mM (1*RS*,2*RS*)-homo-ACPD caused a right-ward shift of the dose–response curve for (*S*)-Glu (Fig. 3A). Using the Gaddum equation a $K_B$ value of 391 μM was determined for (1*RS*,2*RS*)-homo-ACPD (Table 1). (1*S*,3*R*)-homo-ACPD and (1*R*,3*R*)-homo-ACPD both displayed significant agonist activity when tested in 1 mM (Fig. 2B). When examining the

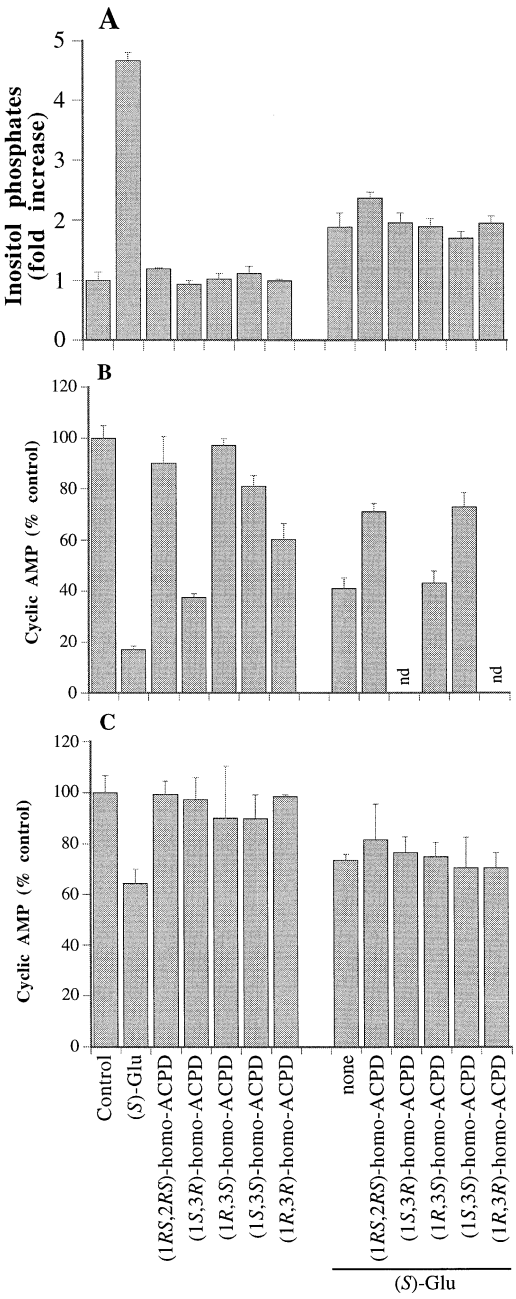


Fig. 2. Agonist activities of (*S*)-Glu and of homo-ACPD's at CHO cells expressing (A) $mGlu_{1\alpha}$, (B) $mGlu_2$ and (C) $mGlu_{4a}$. In agonist assays $mGlu_{1\alpha}$-expressing cells were incubated with ligand at a concentration of 1 mM for 20 min. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 20 min in the presence of 10 μM (*S*)-Glu. Total IP formation was determined by an ion-exchange assay and the fold increase in IP level calculated compared to control cells (incubated in buffer only). In agonist assays $mGlu_2$ and $mGlu_{4a}$-expressing cells were incubated with ligands (1 mM) for 10 min in the presence of 10 μM forskolin. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 10 min in the presence of 20 μM ($mGlu_2$) or 50 μM ($mGlu_{4a}$) (*S*)-Glu and 10 μM forskolin. Cyclic AMP levels were measured by a RIA assay and expressed as percent of cyclic AMP level in control cells (incubated in buffer only). Data are the means (±SD) of representative experiments performed in triplicate.

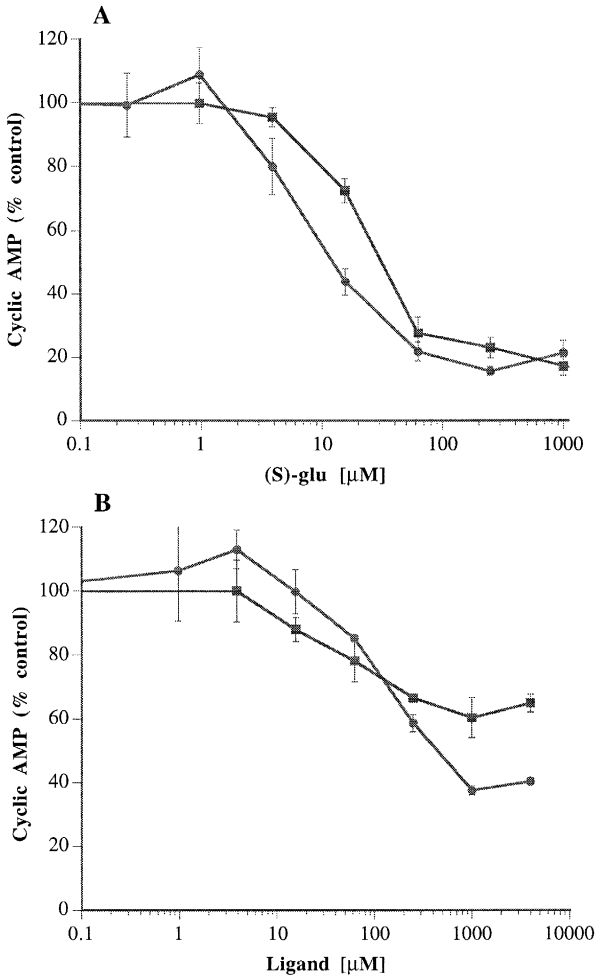


Fig. 3. (A) Dose–response curves of (*S*)-Glu in the absence (●) or presence (■) of 1 mM (1*RS*,2*RS*)-homo-ACPD at $mGlu_2$-expressing cells. (B) Dose–response curves of (1*S*,3*R*)-homo-ACPD (●) and (1*R*,3*R*)-homo-ACPD (■) at $mGlu_2$-expressing cells. For further details, see legend for Fig. 2.

dose–response curves (Fig. 3B) for (1*S*,3*R*)-homo-ACPD and (1*R*,3*R*)-homo-ACPD $EC_{50}$ values of 122 μM and 105 μM, respectively, were obtained (Table 1). As seen in Fig. 3 and Table 1 both compounds were partial agonists with intrinsic activities of 79% and 47%, respectively, compared to the maximal response of (*S*)-Glu. When tested at 1 mM concentration (1*S*,3*S*)-homo-ACPD was both able to decrease the cyclic AMP level by 27 ± 4% compared to the maximal response of (*S*)-Glu and to antagonize partially the (*S*)-Glu induced responses. This indicates that (1*S*,3*S*)-homo-ACPD is a partial agonist, however, the response was too weak to measure a reliable $EC_{50}$ value. Finally, (1*R*,3*S*)-homo-ACPD was found to be inactive at the $mGlu_2$ receptor (Fig. 2).

No effects of the five homo-ACPD's was observed at 1 mM concentration when tested as agonists or antagonists

Table 1
Pharmacological activity of ligands at the mGlu receptor subtypes expressed in CHO cells

| | $EC_{50}$ (μM) [a] (% of maximal (*S*)-glu response) | | |
|---|---|---|---|
| | $mGlu_{1\alpha}$ | $mGlu_2$ | $mGlu_{4a}$ |
| (*S*)-glu | 19 ± 2 (100%) | 7.7 ± 0.7 (100%) | 21 ± 2 (100%) |
| (*S*)-2-aminoadipic acid [b] | > 1000 | 35 ± 1 | > 3000 |
| (1*SR*,3*RS*)-ACPD | 121 ± 6 (83 ± 11%) | 11 ± 1 (105 ± 1%) | ~ 1000 [c] |
| (1*RS*,2*RS*)-homo-ACPD | > 1000 | 391 ± 89 [d] | > 1000 |
| (1*S*,3*R*)-homo-ACPD | > 1000 | 122 ± 49 (79 ± 4%) | > 1000 |
| (1*R*,3*S*)-homo-ACPD | > 1000 | > 1000 | > 1000 |
| (1*S*,3*S*)-homo-ACPD | > 1000 | p.a. (27 ± 4%) | > 1000 |
| (1*R*,3*R*)-homo-ACPD | > 1000 | 105 ± 36 (47 ± 9%) | > 1000 |

[a] Mean ± standard error of mean of at least three independent experiments.
[b] From Bräuner-Osborne et al. (1996).
[c] Estimated from partial dose–response curves.
[d] $K_B$ value calculated using the Gaddum equation. p.a., partial agonist, response too weak to measure a reliable $EC_{50}$ value.

at ionotropic Glu receptors using the cortical wedge preparation of rat neocortex (data not shown).

## 4. Discussion

### *4.1. Partial agonism by analogues of (1,3)-homo-ACPD*

Previously we have found that compounds with different chain lengths can afford subtype selective ligands for excitatory amino acid receptors. It has been shown that (*S*)-2-aminoadipic acid, the longer analogue of (*S*)-Glu, is a selective $mGlu_2$ receptor agonist (see Table 1) (Thomsen et al., 1994; Bräuner-Osborne et al., 1996). Furthermore, the 3-hydoxyisoxazole bioisosteres of aspartate, Glu and 2-aminoadipic acid ((*RS*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA), AMPA and (*RS*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid (homo-AMPA), respectively) are selective NMDA, AMPA and $mGlu_6$ receptor agonists, respectively (Bräuner-Osborne et al., 1996). These observations indicate that the design of analogues of (*S*)-2-aminoadipic acid could be a useful approach to obtain mGlu receptor subtype selective ligands. In agreement with this hypothesis the three active enantiomers of (1,3)-homo-ACPD, which are conformationally restricted analogues of 2-aminoadipic acid, selectively activated the $mGlu_2$ receptor, being inactive at $mGlu_{1\alpha}$ and $mGlu_{4a}$. Thus, the (1,3)-homo-ACPD enantiomers possess greater subtype selectivity than the parent compound ACPD, which also activates group I (Knöpfel et al., 1995; Schoepp et al., 1995) and group III receptors (Gomeza et al., 1996). However, since pharmacological deviations within the groups have been reported (Bräuner-Osborne et al., 1996; Saugstad et al., 1997) it is desirable to test the four isomers at all eight mGlu receptor subtypes in order to fully establish the selectivity profile of the compounds. Unfortunately these are not all available at present to us.

Interestingly (1*S*,3*R*)-homo-ACPD, (1*R*,3*R*)-homo-ACPD and (1*S*,3*S*)-homo-ACPD were all partial agonists at the $mGlu_2$ receptor with decreasing levels of intrinsic activity. To our knowledge these are the first partial agonists reported at the $mGlu_2$ receptor. Very recently, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-4-APDC) and LY354740, both analogues of ACPD, have been reported to be potent and selective $mGlu_2$ receptor agonists (Schoepp et al., 1996, 1997). These results further underline the importance of ACPD as a lead towards the design of new selective mGlu receptor ligands.

### *4.2. Competitive antagonism of (1RS,2RS)-homo-ACPD*

We also tested the pharmacology of (1*RS*,2*RS*)-homo-ACPD, which is a conformationally restricted analogue of (*S*)-Glu. Like the (1,3)-homo-ACPD's this compound was selective for the $mGlu_2$ receptor, being inactive at $mGlu_{1\alpha}$ and $mGlu_{4a}$ though with antagonist activity. As seen in Fig. 3 (1*RS*,2*RS*)-homo-ACPD caused a parallel right-ward shift of the (*S*)-Glu dose–response curve, indicating that it is a competitive antagonist.

### *4.3. Conclusion*

In this study we have reported the discovery of homo-ACPD's as new and selective ligands for the $mGlu_2$ receptor. Interestingly the compounds, which are analogues of ACPD, displayed varying degrees of efficacy spanning from almost full agonists to competitive antagonists. These results support previous studies, which have shown that extension of chain length is an important way of gaining sub-type selectivity within the mGlu receptors. Due to their increased selectivity compared to (1*SR*,3*RS*)-ACPD and their varying degree of efficacy these compounds may be useful pharmacological tools for the mGlu receptors in the central nervous system.

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