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Selective activation of group-II metabotropic glutamate receptors is protective against excitotoxic neuronal death

Giuseppe Battaglia [a], Valeria Bruno [a], Richard T. Ngomba [a], Renato Di Grezia [a], Agata Copani [b], Ferdinando Nicoletti [a,b,*]

[a] *I.N.M. Neuromed, Pozzilli, Italy*

[b] *Institute of Pharmacology, School of Pharmacy, University of Catania, Viale A. Doria, 6, 95125 Catania, Italy*

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Abstract

Aminopyrrolidine-2*R*,4*R*-dicarboxylated (2*R*,4*R*-APDC) has recently been introduced as a potent and highly selective agonist of metabotropic glutamate (mGlu) receptor subtypes $mGlu_2$ and $-_3$. In murine cortical cultures containing both neurons and astrocytes, 2*R*,4*R*-APDC attenuated the delayed neuronal degeneration induced by a 10-min pulse of *N*-methyl-D-aspartate (NMDA). 2*R*,4*R*-APDC was maximally neuroprotective in a range of concentrations (0.1–1 μM) comparable to that reported for the activation of $mGlu_2$ or $-_3$ receptors in heterologous expression systems. The action of 2*R*,4*R*-APDC was sensitive to the $mGlu_{2/3}$ receptor antagonists, (2*S*)-α-ethylglutamate and (2*S*,1*S*′,2*S*′,3*R*′)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine. These results indicate that activation of $mGlu_2$ and/or $-_3$ receptors is sufficient per se to protect neurons against excitotoxic degeneration, and encourage the search for potent, selective and systemically active $mGlu_{2/3}$ receptor agonists as neuroprotective drugs.

Keywords: Aminopyrrolidine-2*R*,4*R*-dicarboxylated; $mGlu_2$ receptor; $mGlu_3$ receptor; Neuroprotection

1. Introduction

Metabotropic glutamate (mGlu) receptors form a family of eight subtypes, which have tentatively been classified into three groups on the basis of sequence homology, pharmacological profile of activation, and transduction pathways. Group-I includes $mGlu_1$ and $-_5$ receptors, which are coupled to polyphosphoinositide hydrolysis; group-II ($mGlu_2$ and $-_3$) and group-III ($mGlu_4$, $-_6$, $-_7$ and $-_8$) receptors are negatively coupled to adenylyl cyclase activity in heterologous expression systems (Nakanishi, 1992; Pin and Duvoisin, 1995). In vitro and in vivo studies indicate that activation of group-II mGlu receptors by (2*S*,2*R*′,3*R*′)-2-(2′,3′-dicarboxyxylopropyl) glycine (DCG-IV), (2*S*,1*S*′,2*S*′)-2-(carboxycyclopropyl)glycine (L-CCG-I) or (*S*)-4-carboxy-3-hydroxyphenylglycine (4C3HPG) protects neurons against excitotoxic death, as well as against apoptosis induced by oxygen–glucose deprivation, β-amyloid peptide or staurosporine (reviewed by Nicoletti et al., 1996). However, the protective role of group-II mGlu receptors has recently been questioned, based on the evidence that the potent agonist (+)-2-aminobicyclo[3.1.0.]hexane-2,6-dicarboxylate (LY354740; Schoepp et al., 1997a) is neuroprotective, but only at concentrations 2–3 orders of magnitude higher than its EC50 value in clones overexpressing $mGlu_2$ or $-_3$ receptors (Kingston et al., 1997). It has been argued that neuroprotection is mediated instead by $mGlu_8$ receptors, which can be recruited by high concentrations of LY354740 (Schoepp et al., personal communication). The same neuroprotective activity of DCG-IV, L-CCG-I or 4C3HPG may in principle be mediated by mechanisms other than the activation of group-II mGlu receptors. Accordingly, micromolar concentrations of DCG-IV activate NMDA receptors (Ishida et al., 1993; Hayashi et al., 1993) and antagonize $mGlu_7$ receptors (Schoepp et al., 1997b); L-CCG-I activates group-I and -III mGlu receptors (Hayashi et al., 1992; Schoepp et al., 1997b); and 4C3HPG behaves as a $mGlu_{1a}$ receptor antagonist (Brabet et al., 1995). To examine whether the activation of group-II mGlu receptors is sufficient to afford neuroprotection, we examined the protective activity of the novel and highly selective $mGlu_{2/3}$ receptor agonist aminopyrrolidine-2*R*,4*R*-dicarboxylate (2*R*,4*R*-APDC; Schoepp et al., 1996) against NMDA toxicity in mixed cultures of mouse cortical cells.

* Corresponding author. Tel./Fax: +39-95-337167.

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2. Materials and methods

2.1. Mixed cortical cultures

Mixed cortical cell cultures containing both neurons and astrocytes were prepared from fetal mice at 14–16 days of gestation, as described by Rose et al. (1992). In brief, dissociated cortical cells were plated in 15-mm multiwell vessels (Falcon Primaria, Lincoln Park, NY) on a layer of confluent astrocytes (7–14 days in vitro), using a plating medium of Minimal Essential Medium (MEM)-Eagle's salts (supplied glutamine-free) supplemented with 5% heat-inactivated horse serum, 5% fetal bovine serum, glutamine (2 mM), glucose (21 mM), and $NaHCO_3$ (25 mM). Cultures were kept at 37°C in a humidified 5% CO_2 atmosphere. After 3–5 days in vitro, non-neuronal cell division was halted by a 1 to 3-day exposure to 10 μM cytosine arabinoside, and cultures were shifted to a maintenance medium identical to the plating medium but lacking fetal bovine serum. Subsequent partial medium replacement was carried out twice a week. Only mature cultures (13–14 days in vitro) were used in our experiments.

Glial cell cultures used as a support for mixed cultures were prepared as described by Rose et al. (1992) from postnatal mice (1–3 days after birth).

2.2. Exposure of mixed cultures to excitatory amino acids and assessment of neuronal injury

Brief exposure to *N*-methyl-D-aspartate (NMDA, 10 min) in the absence or presence of mGlu receptor agonists and/or antagonists was carried out in mixed cortical cultures at room temperature in a HEPES-buffered salt solution containing (in mM): 120 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.8 $CaCl_2$, 20 HEPES, 15 glucose. After 10 min, the drugs were washed out, and the cultures were incubated at 37°C for the following 24 h in MEM-Eagle's supplemented with 25 mM $NaHCO_3$ and 21 mM glucose. In another set of experiments, 2*R*,4*R*-APDC was added to cultures immediately after the NMDA pulse and remained in the medium for the following 24 h.

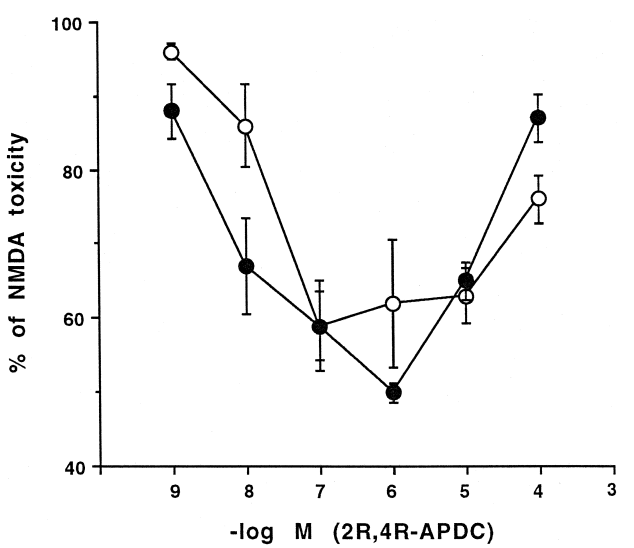

Fig. 1. Concentration-dependent attenuation of NMDA toxicity by 2*R*,4*R*-APDC in mixed cortical cultures. 2*R*,4*R*-APDC was applied to the cultures either in combination with NMDA (closed circles) or immediately after the NMDA pulse (open circles). Results are expressed as percentages (±S.E.M.) of the toxicity of NMDA and were calculated from 6–12 determinations.

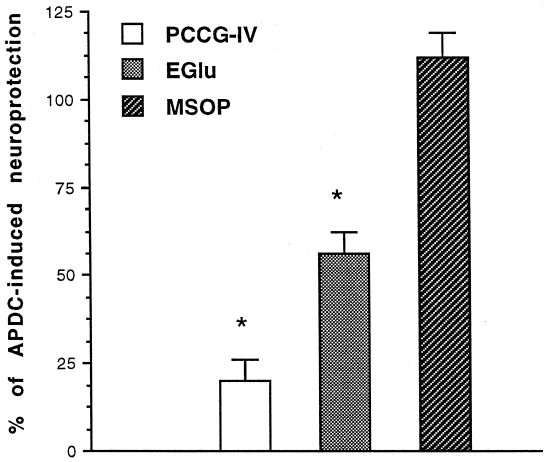

Fig. 2. The neuroprotective activity of 2*R*,4*R*-APDC (1 μM) against NMDA in cultured cortical cells is antagonized by EGlu (100 μM) or PCCG-IV (20 μM), but not by MSOP (100 μM). 2*R*,4*R*-APDC and/or mGlu receptor antagonists were applied in combination with NMDA. Results are expressed as percentages (±S.E.M.) of 2*R*,4*R*-APDC-induced neuroprotection, and were calculated from six determinations. $* P < 0.01$ (one-way ANOVA + Fisher PLSD), if compared with 2*R*,4*R*-APDC alone. None of the antagonists influenced NMDA toxicity per se (not shown).

Neuronal injury was estimated in all experiments by examination of the cultures by phase-contrast microscopy 24 h after the insult, when the process of cell death was largely complete. Neuronal damage was quantitatively assessed in all experiments by estimation of dead neurons by Trypan-blue staining. Stained neurons were counted from three random fields per well.

2.3. Materials

2*R*,4*R*-APDC was kindly provided by Dr. Darryle Schoepp (Ely Lilly, Indianapolis, IN); DCG-IV, (2*S*)-α-ethylglutamate (EGlu), (2*S*,1*S*′,2*S*′,3*R*′)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCG-IV), (*RS*)-α-methylserine-*O*-phosphate (MSOP) and NMDA were purchased from Tocris Cookson (Langford, UK); all other drugs or chemicals were purchased from Sigma (Milano, Italy).

3. Results

In mixed cortical cultures, a 10-min pulse with 100 μM NMDA produced a delayed degeneration of 70–80% of

the neuronal population, as estimated by Trypan blue staining. 2*R*,4*R*-APDC applied either in combination with NMDA or immediately after the NMDA pulse attenuated neuronal degeneration in a concentration-dependent fashion. Maximal neuroprotection was observed at concentrations of 2*R*,4*R*-APDC ranging from 0.1 to 10 μM, whereas the drug partially lost its activity at 100 μM (Fig. 1). The neuroprotection provided by 1 μM 2*R*,4*R*-APDC was substantially reduced by the group-II mGlu receptor antagonists, PCCG-IV (20 μM) or EGlu (100 μM), but not by the preferential group-III mGlu receptor antagonist, MSOP (100 μM) (Fig. 2). In all these experiments, neither 2*R*,4*R*-APDC nor any of the mGlu receptor antagonists influenced neuronal viability per se (not shown).

4. Discussion

The neuroprotective activity of group-II mGlu receptor agonists was initially described in mixed cultures of cortical cells (Bruno et al., 1994; Buisson et al., 1994) and then confirmed in different in vitro or in vivo models of excitotoxicity (Shinozaki, 1994; Ambrosini et al., 1995; Bruno et al., 1995, 1997, 1998; Buisson and Choi, 1995; Buisson et al., 1996; Copani et al., 1995; Orlando et al., 1995; Turetsky et al., 1995). Neuroprotection is generally thought to be due to the inhibition of glutamate release by presynaptic $mGlu_2$ or $-_3$ receptors (reviewed by Pin and Duvoisin, 1995). Microdyalisis studies have shown that $mGlu_{2/3}$ receptor agonists substantially reduce the release of glutamate evoked by depolarizing agents without affecting the basal release (Battaglia et al., 1997). Hence, $mGlu_{2/3}$ receptor agonists may be considered as promising neuroprotective drugs, because they should prevent the deleterious effects of excessive glutamate release without causing sedation, ataxia or other side effects related to an impairment of excitatory synaptic transmission. However, recent evidence casts doubt on the real effectiveness of $mGlu_{2/3}$ receptor agonists as neuroprotective agents (see Section 1). We now report the neuroprotective activity of the recently synthesized, highly selective group-II mGlu receptor agonist, 2*R*,4*R*-APDC (Schoepp et al., 1996). This drug was effective against NMDA toxicity in cortical cultures. The potency of 2*R*,4*R*-APDC in cultures was comparable to that reported for the activation of $mGlu_2$ and $-_3$ receptors (see Schoepp et al., 1996, 1997b), and its neuroprotective activity was attenuated by the selective group-II mGlu receptor antagonists, EGlu (Thomas et al., 1996) or PCCG-IV (Thomsen et al., 1996). It is intriguing that the protective activity tended to vanish with increasing concentrations of 2*R*,4*R*-APDC, because the drug does not interact with metabotropic or ionotropic glutamate receptors other than $mGlu_2$ or $-_3$ (Schoepp et al., 1996, 1997b). It is possible that (i) 2*R*,4*R*-APDC is metabolized by neurons or astrocytes to generate a toxic compound that counterbalances the protective activity of the parent drug; (ii) the pharmacological profile of the compound includes additional unknown receptors; or (iii) high concentrations of 2*R*,4*R*-APDC reduce γ-amino-*n*-butyric acid release (see Pin and Duvoisin, 1995), thus removing a major constraint to the development of excitotoxic death.

The present data demonstrate that selective activation of group-II mGlu receptors is sufficient to afford significant neuroprotection. How can these results be reconciled with the low potency of LY354740 as neuroprotectant in pure neuronal cultures (Kingston et al., 1997)? One possible explanation is that the presence of astrocytes enables the neuroprotective activity of group-II mGlu receptor agonists. Accordingly, we have recently shown that activation of glial $mGlu_3$ receptors in culture renders the medium neuroprotective against NMDA toxicity (Bruno et al., 1997, 1998). This particular form of glial-neuronal interaction is sensitive to inhibitors of protein synthesis, suggesting that astrocytes respond to the activation of $mGlu_3$ receptors by synthesizing and releasing a proteic neuroprotective factor (Bruno et al., 1997). Thus, any difference in the potency or efficacy of group-II mGlu receptor agonists as neuroprotectants may simply reflect the number of astrocytes present in the culture system.

We encourage the search for potent, selective and systemically active group-II mGlu receptor agonists as neuroprotective drugs of potential application in the therapy of acute or chronic neurodegenerative disorders.

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