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Short communication

(1*S*,3*R*)-ACPD, a metabotropic glutamate receptor agonist, enhances damage after global ischaemia

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Abstract

There are opposing results in the literature concerning the influence of (1*S*,3*R*)-ACPD [(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate: group I/II metabotropic glutamate receptor agonist) on neurodegeneration, showing both enhancement and reduction of damage. We examined the effects of (1S,3R)-ACPD, given in various application schedules, on global ischaemia in gerbils. The most pronounced effect was a significant increase of hippocampal damage when the drug was applied at 20 mg/kg i.p. pre ischaemia. All other experiments with lower concentrations (0.02–2 mg/kg), other time schedules (post-ischaemic application) or co-application of a selective group I metabotropic glutamate receptor antagonist (4-CPG: (*S*)-4-carboxyphenylglycine), had no influence on neuronal density.

Keywords: (1S,3R)-ACPD [(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate; Global ischaemia; Metabotropic glutamate receptor; Hippocampus; (Gerbil)

1. Introduction

Metabotropic glutamate receptors are a relatively new subclass of excitatory amino acid receptors. Currently, eight cloned metabotropic glutamate receptor subtypes are known ($mGlu_{1-8}$ receptors) which are divided into three groups (group I: $mGlu_{1,5}$ receptor; group II: $mGlu_{2,3}$ receptor; group III: $mGlu_{4,6,7,8}$ receptor). They function through several signal transduction pathways, such as cAMP, protein kinase C, inositol phosphate (release of intracellular Ca^{2+}), ion channel flux and phospholipase D. In general, it is assumed that activation of group I metabotropic glutamate receptors increases excitation whereas agonists of metabotropic glutamate receptors of group II and III lead to a depression of synaptic transmission (for an overview see Conn and Pin, 1997). However, the role of metabotropic glutamate receptor ligands in the pathophysiology of excitotoxic/ischaemic neurodegeneration is far from being clear. There are only a few reports on this topic which involve information about the in vivo situation. Antagonists of group I metabotropic glutamate receptors seem to have a weak neuroprotective potency (Riedel et al., 1996). The same is true for pure agonists of group II metabotropic glutamate receptors (Bond et al., 1998). Drugs which cover both effects simultaneously (group I antagonist/group II agonist) seem to lead to the most convincing results, demonstrated by the successful treatments of global and focal ischaemia by L-AP3 (L(+)-2-amino-3-phosphonopropionic acid) and (*S*)-4C3HPG [(*S*)-4-carboxy-3-hydroxyphenylglycine] (Maginn et al., 1995; Henrich-Noack et al., 1998; Rauca et al., in press). However, the role of (1*S*,3*R*)-ACPD [(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate], a group I and II agonist, in the pathophysiology of cerebral ischaemia is highly controversial. Functional experiments suggest that in theory this compound might enhance the development of neuronal death after excitotoxic challenges: (1*S*,3*R*)-ACPD can increase the concentration of intracellular Ca^{2+} (Linden et al., 1994) and several labs have shown that it enhances NMDA (*N*-methyl-D-aspartate) responses (Harvey and Collingridge, 1993; Pisani et al., 1997; Mannaioni et al., 1996). In line with this hypothesis are several reports which show that the drug can increase NMDA-induced neuronal damage in vitro and in vivo (McDonald and Schoepp, 1992; Bruno et al., 1995) and that it induces seizures in rats and mice (McDonald et al., 1993; Tizzano et al., 1995). However, surprisingly, there exist also many

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reports about *neuroprotective* actions of (1*S*,3*R*)-ACPD: this drug attenuates NMDA-induced neurotoxicity in cortical cultures (Koh et al., 1991; Birrell et al., 1993) and slices (Pizzi et al., 1996), reduces anoxic/hypoxic and nitric oxide induced cell death in hippocampal cultures (Maiese et al., 1996) and slices (Opitz and Reymann, 1993) and one report showed even in vivo a neuroprotective effect after focal ischaemia (Chiamulera et al., 1992). With the present investigation we tried to further elucidate the influence of (1*S*,3*R*)-ACPD on neurodegeneration in vivo and examined the effect of this compound on delayed neuronal death in the hippocampal CA1 region after transient global ischaemia in gerbils.

2. Material and methods

Male Mongolian gerbils (60–90 g; Tumblebrook farm strain) were housed with a 12 h light–dark cycle and given food and water ad libitum. To induce ischaemia, the animals were anaesthetized with 2.5-3% halothane in a mixture of nitrous oxide/oxygen (70:30). Both common carotid arteries were exposed by a ventral midline incision and separated carefully from the adjacent veins and nerves. The vessels were clamped by surgical clips and halothane was reduced to 0.75%. After 5 min, the clips were removed and restoration of blood flow was visually confirmed. The operation was performed on a soft heating mat and animals were kept normothermic. Afterwards, the wound was treated with lidocaine gel and sutured. The animals were kept under a heating lamp at 30°C environmental temperature until they regained consciousness. After the survival period of seven days, the brains were removed, fixed by immersion and embedded in paraffin or the brains were quickly frozen in liquid nitrogen. Hippocampal slices (12 μm) were cut on a microtome/cryotome and life/death staining was performed with toluidine blue/fuchsin acid. Healthy neurones were counted in a blinded manner on a microscope within a 500 mm area of the hippocampal region. If i.c.v. injection was necessary (experiment with 4-CPG [(*S*)-4-carboxyphenylglycine]), eight days before performing ischaemia the animals were anaesthetized with pentobarbital (80 mg/kg) and fixed in a stereotaxic device. Cannulae (diameter 0.6 mm) were inserted in the lateral ventricle (0.6 mm posterior to bregma, 1.2 mm lateral to midline, 2.6 mm from skull). The positions of the cannulae were verified by histochemistry.

(1*S*,3*R*)-ACPD was injected i.p. in all experiments. Controls were treated identically as drug-treated animals but with vehicle alone. Each group consisted of 8–10 animals. Data are mean ± S.E.M..

3. Results

(3.1) (1*S*,3*R*)-ACPD was applied i.p. in a concentration of 20 mg/kg 20 min before induction of ischaemia. This

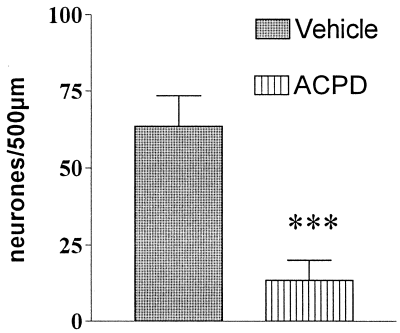

Fig. 1. (1*S*,3*R*)-ACPD decreases the number of surviving pyramidal cells after transient global ischaemia. The drug was applied i.p. 20 min before ischaemia in a dose of 20 mg/kg. Controls were treated identically with vehicle alone. The bars represent the number of morphologically healthy neurones within a 500 μm area of hippocampal the CA1 layer. Values are given as mean ± S.E.M.; $n = 10$ in each group; different from control: *** $P < 0.001$ by Mann–Whitney, *U*-test.

treatment caused a pronounced deterioration of neuronal damage (numbers are always given as healthy neurones per 500 μm CA1 region): control: 63 ± 10 vs. (1*S*,3*R*)-ACPD: 14 ± 7; $P < 0.001$, Mann–Whitney, *U*-test; Fig. 1).

(3.2) Also, lower doses were tested, but showed no effect: 2 mg/kg: control: 23 ± 14 vs. (1*S*,3*R*)-ACPD: 17 ± 4; 0.2 mg/kg: control: 58 ± 23 vs. (1*S*,3*R*)-ACPD: 75 ± 20; 0.02 mg/kg: control: 36 ± 16 vs. (1*S*,3*R*)-ACPD: 52 ± 19).

(3.3) Furthermore, 20 mg/kg (1*S*,3*R*)-ACPD were injected i.p. 10 min after clamping both common carotid arteries. There was a slight increase of neuronal damage (control: 23 ± 14 vs. (1*S*,3*R*)-ACPD: 2 ± 1, $P = 0.055$; Mann–Whitney, *U*-test). Also, lower doses (2 mg/kg) did not enhance neuronal survival.

(3.4) Additionally, we combined injection of 4-CPG (0.2 mg/animal, 20 min pre ischaemia, i.c.v.) with application of (1*S*,3*R*)-ACPD (i.p.; 2 mg/kg; 5 min after reperfusion). There was no difference in the number of healthy hippocampal neurones (control: 53 ± 20 vs. (1*S*,3*R*)-ACPD: 59 ± 18).

(3.5) Finally, the drug was injected (20 mg/kg) once 24 h before clamping both common carotid arteries (ACPD1 ×) or three times before the challenge (interval between each injection: 12 h; last injection 24 h before ischaemia: ACPD3 ×). But also this schedule did not lead to a significant increase in surviving neurones (control: 17 ± 12 vs. ACPD1 ×: 36 ± 19 vs. ACPD3 ×: 20 ± 17).

4. Discussion

Our first experimental approach (3.1) indicates that activation of group I/II metabotropic glutamate receptors increases neuronal injury after transient global ischaemia (Fig. 1). We also investigated whether this amount of (1*S*,3*R*)-ACPD per se can cause neurodegeneration, but

we did not see any difference in the neuronal density of the hippocampus in comparison to the vehicle-treated group when 20 mg/kg were injected i.p. without performing ischaemia (data not shown). However, if the drug is given i.c.v. in a dose of 20 nmol it causes death of pyramidal cells in the hippocampus of Wistar rats (Manahan-Vaughan et al., submitted).

To check whether the effects of (1*S*,3*R*)-ACPD might be crucially dependent on the applied dose, we tested a range of lower concentration (0.02–2 mg/kg), but, also ,these conditions never caused any neuroprotective effect (3.2.).

Suzuki et al. (1996) reported that (1*S*,3*R*)-ACPD induces seizures when injected in the amygdala of naive rats. But interestingly the application of the compound results in a marked delay and suppression of seizures in kindled rats. This suggests that (1*S*,3*R*)-ACPD might have bidirectional effects (excitatory and inhibitory) on neurones, depending on the condition of the cell. Since the drug showed anti-excitotoxic effects when the rats were kindled *before* application, we hypothetized that (1*S*,3*R*)-ACPD could also reduce excitotoxicity when ischaemia has been induced *before* the drug is injected. Therefore the compound was also tested in a post-ischaemic application schedule (3.3). However, in our hands this treatment also did not protect CA1 neurones, but rather led to a tendency towards deterioration. When publishing the neuroprotective effect of (1*S*,3*R*)-ACPD on hypoxic/hypoglycemic slices, Small et al. (1996) suggested that the in vitro effect of this compound on CA1 neurones involves metabotropic glutamate receptors which are negatively linked to cAMP (i.e., group II mGlu receptors). To prove the hypothesis that (1*S*,3*R*)-ACPD may exert neuroprotection via its group II metabotropic glutamate receptor agonistic potency, and that its simultaneous activation of group I rather counteracts this effect, we injected the metabotropic glutamate receptor group I antagonist 4-CPG (the applied dose per se has no influence on ischaemic damage; Henrich-Noack and Reymann, 1997) and afterwards (1*S*,3*R*)-ACPD (3.4). The EC_{50} values of 4-CPG and (1*S*,3*R*)-ACPD are in the same range (Conn and Pin, 1997) and from our experience, i.c.v. injections of drugs in micromolar concentrations lead to higher brain tissue concentrations than i.p. injections of a few milligrams. Therefore, theoretically, the action of (1*S*,3*R*)-ACPD on group I receptors should be totally blocked. This explains why we could not see any tendency towards an increased number of dead neurones; however, there was also definitely no protection.

In contrast to the hypothesis drawn from the paper of Suzuki et al. (1996) (post-ischaemic application could be protective) in vitro data from our laboratory indicated that activation of metabotropic glutamate receptor group I may change the physiological status of neurones towards an increased resistance against oxygen deprivation and thereby can reduce damage when the ligand is applied *before* induction of ischaemia (Schröder et al., in press). Therefore, we tried to induce a kind of chemical preconditioning with (1*S*,3*R*)-ACPD (3.5) but could also not find neuroprotection under our in vivo circumstances.

In summary, the present study demonstrates that (1*S*,3*R*)-ACPD amplifies ischaemia-induced neuronal degeneration in the hippocampal CA1 region. We still cannot exclude that the drug might lead to neuroprotection when applied in a further schedule, however, injection of the compound according to the schedule of Chiamulera et al. (who found protection after focal ischaemia in mice with post ischaemic injection of 20 mg/kg t-ACPD) has no such successful effect in the model of transient global ischaemia in gerbils. In our hands the main feature of this agonist of group I/II metabotropic glutamate receptors is a pronounced potency to deteriorate hippocampal damage.

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