Neuropharmacology 102 (2016) 276–294

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Neuroprotective potential of the group III mGlu receptor agonist ACPT-I in animal models of ischemic stroke: *In vitro* and *in vivo* studies

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ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
7 November 2015
Accepted 24 November 2015
Available online 2 December 2015

Keywords:
OGD
MCAO
Calpains
Glutamate
cAMP/PKA pathway
CatWalk

ABSTRACT

In the present study, we investigated the effect of ACPT-I [(1*S*, 3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid], a blood-brain-barrier permeable agonist of group III mGlu receptor, against oxygen-glucose deprivation (OGD)-evoked neuronal cell death in primary neuronal cell cultures and in the model of transient middle cerebral artery occlusion (MCAO) in rats. We found that ACPT-I (1–200 μM) in a concentration- and time-dependent way attenuated the OGD-induced neuronal cell damage, being also effective after a delayed application (30 min after OGD). The neuroprotective effects of ACPT-I were blocked by the group III mGlu receptor antagonist, (*RS*)-alpha-cyclopropyl-4-phosphonophenyl glycine (CPPG), and by the activator of cAMP-dependent PKA, 8-Bromo-cAMP, but not by an inhibitor of PI-3-K signaling pathway. Moreover, ACPT-I attenuated the OGD-induced calpain activity and glutamate release. In the *in vitro* study, we also demonstrated the neuroprotective potential of mGluR4 positive allosteric modulators (PAMs), PHCCC (30 μM) and VU0155041 (10 and 30 μM) and synergism in neuroprotective action of low concentrations of ACPT-I and mGluR4 PAMs suggesting an important role of mGluR4 activation in prevention of ischemic neuronal cell death. In the rat MCAO model, we demonstrated that ACPT-I (30 mg/kg) injected intraperitoneally either 30 min after starting MCAO or 30 min after beginning reperfusion not only diminished the infarction volume by about 30%, but also improved selected gait parameters (CatWalk analysis) and the mobility of animals in the open field test. In conclusion, our results indicate that ACPT-I may be not only neuroprotective against ischemic neuronal damage but may also diminish the postischemic functional deficits.

1. Introduction

Ischemic stroke is a major cause of morbidity and mortality worldwide (Grupke et al., 2015). The cessation or critical reduction in blood flow that occurs during acute stroke results in deprivation of the oxygen and glucose supplies, which can produce a local brain ischemia and injury. It is well established that excitotoxicity, a type of neurotoxicity evoked by elevated extracellular glutamate level is a primary contributor to ischemic neuronal death (Choi, 1994; Lai et al., 2014; Olney, 1978; Olney and Ishimaru, 1999; Puyal et al., 2013). Cerebral ischemia elicits a massive increase in extracellular glutamate level because of enhanced efflux from the presynaptic terminals and reduction of uptake, causing the activation of several glutamate receptors (Nishizawa, 2001). Glutamate, the main excitatory neurotransmitter in the mammalian brain (Headley and Grillner, 1990), mediates its effect on cells via activation of ionotropic (iGluRs) (NMDA, AMPA, and kainate receptors) and metabotropic (mGluRs) glutamate receptors (Nakanishi et al., 1998). Overstimulation of glutamate receptors (particularly NMDARs, and also AMPA/kainate receptors) induces an increase in intracellular Ca^{2+} concentrations, release of K^{+} into the extracellular space, and cell swelling due to the passive movement of water with Na^{+} influx. Consequently, the massively increased intracellular second

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http://dx.doi.org/10.1016/j.neuropharm.2015.11.025
0028-3908/

messenger Ca^{2+} triggers numerous deleterious processes, including free radical formation, membrane degradation, mitochondrial dysfunction, inflammation, activation of various enzymes (e.g. caspases, calpains, liposomal proteases, and endonucleases), and DNA fragmentation which finally lead to neuronal cell death by necrosis and/or apoptosis (Choi, 1994; Grammer et al., 2008; Lipton, 1999; Minnerup et al., 2012; Prass and Dirnagl, 1998). Within the ischemic cascade, many molecular targets can be pharmacologically modulated to produce neuroprotection and glutamate receptors represent one of such targets. Although various NMDAR or AMPA/kainate receptor antagonists have been studied and found to be neuroprotective in animal stroke models (Belayev et al., 1995; Gill et al., 1987; O'Neill et al., 1998; Simon et al., 1984), clinical trials have been unsuccessful because of the narrow therapeutic window, the occurrence of undesirable side effects, or the lack of efficacy (Grupke et al., 2015; Ikonomidou and Turski, 2002; Liu et al., 2012; Muir and Lees, 1995; Neuhaus et al., 2014; Xu and Pan, 2013). Thus, it has been postulated that an indirect inhibitory modulation of the glutamatergic transmission by the compounds acting on mGlu receptors might be a more promising strategy of neuroprotection (Byrnes et al., 2009; Lea and Faden, 2003) as it could be devoid of severe side effects (Bruno et al., 2001; Hovelsø et al., 2012; Nicoletti et al., 1996).

The mGluRs belong to the family of G-protein coupled receptors and are classified into three groups (I–III) on the basis of their sequence homology, signal transduction pathways and pharmacological profiles (Ferraguti and Shigemoto, 2006; Pin and Duvoisin, 1995). Group I mGluRs (containing mGlu1 and mGlu5) are positively coupled to phospholipase C through G_q protein and their activation leads to phosphoinositide hydrolysis and intracellular mobilization of Ca^{2+} ions. Receptors of group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) are negatively coupled to adenylyl cyclase through G_i/G_o proteins, and their activation leads to the inhibition of the cAMP formation (Conn and Pin, 1997; Spooren et al., 2003).

In the present paper, we focused on the compound activating group III mGlu receptor subtypes. These receptors are distributed throughout different regions of the central nervous system (CNS) and are localized predominantly on presynaptic terminals of glutamatergic and GABAergic neurons, where they are involved in the regulation of synaptic transmission (Conn and Pin, 1997). Besides the presynaptic location, the postsynaptic localization of these mGluRs has also been described (Bradley et al., 1996). It has been shown that the activation of presynaptic group III mGlu receptors located on the glutamatergic nerve terminals causes a decrease in glutamate release, thus inhibiting glutamatergic excitatory transmission (Cartmell and Schoepp, 2000; Schoepp, 2001). Hence, it has been suggested that the activation of these receptors may have neuroprotective effects. A number of data have confirmed the neuroprotective properties of group III mGluR agonists against excitotoxicity evoked by NMDA, quinolinic acid, kainate (KA) or homocysteic acid in different animal models *in vitro* (Bruno et al., 2000, 1996; Domin et al., 2014; Gasparini et al., 1999; Iacovelli et al., 2002; Lafon-Cazal et al., 1999) and *in vivo* (Bruno et al., 2000; Domin et al., 2014; Folbergrová et al., 2008; Gasparini et al., 1999). However, little is known about the role of group III mGlu receptor activation in neuroprotection against ischemic brain damage. Up till now, it has been shown that the selective mGlu4 receptor enhancer, PHCCC attenuated the ischemic brain damage in mice (MCAO model) and rats (endothelin-1 model) and mice lacking mGlu4 receptor showed a higher infarct volume after MCAO than their wild-type littermates (Moyanova et al., 2011). Moreover, there are studies showing that transient global ischemia leads to an early increase in mGlu4 receptor mRNA levels in the hippocampus and parietal cortex, with no changes in the transcript of mGlu1, mGlu2, and mGlu5 receptors, and a decrease in mGlu3 receptor mRNA levels (Rosdahl et al., 1994; Iversen et al., 1994). The above-mentioned results indicate that mGlu4 receptors may be attractive targets for neuroprotective therapy in ischemic brain damage. The limited number of studies on the role of group III mGlu receptors in ischemia may be due to a lack of their high-affinity, highly selective and brain-penetrating ligands.

Recent data indicate that the agonist of group III mGluR (1*S*, 3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-I), with preferential affinity for mGlu4, mGlu6 and mGlu8 receptors (Goudet et al., 2008) crosses the blood–brain barrier and is able to penetrate into the brain after intraperitoneal administration (Pałucha-Poniewiera et al., 2008, 2009; Stachowicz et al., 2009). Up until now, there have been very few studies on the neuroprotective effects of ACPT-I in cellular and animal models (Domin et al., 2014; Jantas et al., 2014, 2015).

Therefore, in the present study, we evaluated neuroprotective potential of ACPT-I in primary neuronal cell cultures exposed to oxygen-glucose deprivation (OGD) and in rats after transient middle cerebral artery occlusion (MCAO). We employed the OGD model to investigate the mechanism of neuroprotective action of ACPT-I at the cellular and molecular level, whereas the MCAO model in rats was chosen to confirm *in vivo* neuroprotective potential of ACPT-I as well to study functional improvement (Encarnacion et al., 2011). For the latter purpose, we used the CatWalk system, which automatically calculates gait parameters and the results obtained by this analysis may be comparable to the parameters described in patients (Bederson et al., 1986b; Encarnacion et al., 2011; Mountney et al., 2013).

In the majority of preclinical studies, the drugs were given predominantly before, simultaneously or shortly after the damage, whereas a longer time window is permitted in clinical trials, therefore, in the present study, we applied ACPT-I at different time points, also after the OGD and MCAO induction, which makes our experiments more clinically relevant.

2. Materials and methods

2.1. In vitro study

2.1.1. Chemicals

(1*S*,3*R*,4*S*)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid (ACPT-I), (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG), MK-801 and MDL28170 were from Tocris Bioscience (Bristol, UK). Neurobasal A medium and supplement B27 were from Gibco (Invitrogen, Poisley, UK). The Cytotoxicity Detection Kit and BM Chemiluminescence Western Blotting Kit were from Roche Diagnostic (Mannheim, Germany). Amplex Red Glutamic Acid/Glutamate Oxidase assay kit was from Molecular Probes (Eugene, OR, USA). ABC-peroxidase kit and diaminobenzidine (DAB) were from Vector Laboratories Ltd (Peterborough, UK). Primary antibodies: anti-MAP-2 was from Sigma–Aldrich (St. Louis, MO, USA), anti-spectrin α II (sc-48382) and anti-β-actin (sc-47778) were from Santa Cruz Biotechnology Inc. (CA, USA). Protein markers and appropriate secondary antibody were from Santa Cruz Biotechnology Inc. (CA, USA) or Vector Laboratories Ltd (Peterborough, UK). All other reagents were from Sigma–Aldrich (St. Louis, MO, USA).

2.1.2. Primary neuronal cell cultures

The experiments were conducted on primary cultures of mouse cortical neurons. All the procedures were carried out in accordance with the Local Bioethical Commission Guide for the Care and Use of Laboratory Animals. Neuronal tissues were taken from Swiss mouse embryos at 15/16 day of gestation and were cultivated essentially as described previously (Brewer, 1995; Domin et al.,