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Neuroprotection by group I metabotropic glutamate receptor antagonists in forebrain ischemia of gerbil

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Abstract

Stimulation of group I metabotropic glutamate receptors (mGluR 1 and 5) activates G-protein coupled-phospholipase C (PLC) to release 1,2-diacylglycerol (DAG) and arachidonic acid (ArAc). To elucidate the role of group I mGluR, we tested the effects of (S)-α-methyl-4-carboxy-phenylglycine (MCPG, mGluR 1 and 5 antagonist), 1-aminoindan-1,5-dicarboxylic acid (AIDA, mGluR 1a specific antagonist) and 2-methyl-6-(phenylethynyl) pyridine (MPEP, mGluR 5 antagonist) on ArAc release and neuronal survival after transient forebrain ischemia in gerbils. Ischemia resulted in (a) significant release of ArAc at 1-day reperfusion and (b) significant neuronal death in the hippocampal CA_1 subfield after 6-day reperfusion. MCPG and MPEP decreased ArAc release and also significantly increased neuronal survival. AIDA was less effective in decreasing ArAc release and had no effect on neuronal death. These results suggest that activation of mGluR 5 may be an important pathway in ArAc release and neuronal death after transient ischemia.

Keywords: Arachidonic acid; CA_1 neuronal death; Hippocampus; Apoptosis; Lipid metabolism; 1,2-diacylglycerol; Phospholipases; 2-Methyl-6-(phenylethynyl) pyridine

A major mechanism of neuronal damage during ischemia is the massive release of endogenous glutamate/aspartate [17] and over-stimulation of excitatory amino acid receptors in those brain areas destined to die. Excitotoxic neuronal injury appears to be mediated through stimulation of ionotropic (*N*-methyl-D-aspartate (NMDA)-, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-, and kainate), and metabotropic glutamate receptors (mGluR) [4,15]. Of the three subtypes of mGluR [26], group I (mGluR 1 and 5) are coupled to phospholipase C (PLC) through G-proteins [17]. PLC cleaves phosphatidylinositides to 1,2-diacylglycerol (DAG) which is further hydrolyzed to free fatty acids (FFA) including arachidonic acid (ArAc) [23]. Oxidative metabolism of ArAc has been implicated as a causal factor in ischemic neuronal death [10,20–23].

Antagonists of group I mGluR proved to be neuroprotective [17]. (S)-α-methyl-4-carboxy-phenylglycine (MCPG), a mGluR 1 and 5 antagonist, completely blocked the glutamate-stimulated phosphatidylinositol hydrolysis [18] and provided protection to CA_1 hippocampal neurons [25]. Studies have shown that mGluR 5 is expressed more than mGluR 1 in CA_1 pyramidal neurons [13] and suggested that mGluR 5 might be more involved in ischemic brain injury than mGluR 1 [4]. However, group I mGluR role in central nervous system (CNS) injury has not been clearly defined since a number of studies have not provided conclusive results [3,4,17]. This study evaluated the contribution of mGluR 1 vs. mGluR 5 to ArAc release and hippocampal CA_1 neuronal death after transient global forebrain ischemia in gerbil. The following materials were obtained from the indicated suppliers: chemicals and lipid standards (Sigma Chemicals, St. Louis, MO), MCPG, 1-aminoindan-1,5-dicarboxylic acid (AIDA) and 2-methyl-6-(phenylethynyl) pyridine (MPEP) (Tocris, Ballwin, MO); HPLC grade solvents (Fisher Scientific, Pittsburgh, PA), thin-layer chromatography (TLC) plates (Analtech, Newark, DE).

All surgical procedures were conducted according to the animal welfare guidelines set forth in the NIH Guide for the

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Care and Use of Laboratory Animals (US Department of Health and Human Services Pub 85-23, 1985) and were approved by the University of Wisconsin animal care committee. Male Mongolian gerbils (50–80 g) were anesthetized with 1% halothane in 70:30 N_2O:O_2. Both carotid arteries were exposed by a neck incision, occluded with aneurysm clips for 10-min and reperfused for up to 6 days [20–23]. Brain temperature was measured by means of a thermocouple probe placed in the temporalis muscle. Body and cranial temperatures were maintained at 36.5–37.5°C using a thermostatically controlled water blanket and heating lamp. Physiological variables (mean arterial blood pressure, pO_2 and pCO_2) were monitored. Arterial blood gases were maintained within normal limits (pO_2 75–100 mmHg; pCO_2 25–40 mmHg) [19] for the sham and ischemic groups during the ischemia, and for 3-h post-ischemia reperfusion. Treatment with MCPG, AIDA or MPEP did not affect the physiological variables [22]. MCPG (25 mg/kg i.p.), AIDA (25 mg/kg i.p.), and MPEP (10 mg/kg i.p.) were given to gerbils ($n = 7$ per group) just after the end of ischemia and at 3-h reperfusion. Ischemia causes blood–brain barrier dysfunction quite early after the onset of reperfusion and remains so for long periods, which allows test agents to enter the brain [6,22]. The doses for MCPG, MPEP and AIDA were chosen from previous studies as the minimum doses needed to obtain a significant effect on ArAc release [22]. Treatment with the test agents did not affect the physiological variables. Ischemic controls were treated with vehicle (vhcl. 1 = 0.1 N NaOH adjusted to pH ~7.5 for MCPG and AIDA or vhcl. 2 = 5% DMSO in saline for MPEP). Brains of the anesthetized gerbils were in situ frozen and hippocampi were dissected at 0°C for lipid analysis [20–23].

Brain tissue was extracted with $CHCl_3$:MeOH (1:2, v:v) containing 0.01% butylated hydroxytoluene and 10 nmol of heptadecanoic acid (17:0) as internal standard for FFA [21,23]. FFA and DAG were separated on silica gel G TLC plates using petroleum ether:ether:acetic acid (80:20:1 v/v/v), identified using authentic standards, then converted to methyl esters and analyzed using a Hewlett Packard 6890 gas chromatograph as described [20,21,23]. Quantification was based on external standard calibration with 17:0 as internal standard. Blank TLC regions corresponding to DAG and FFA did not show any GC peaks corresponding to ArAc.

Gerbils were anesthetized 6 days after ischemia and perfused transcardially with buffered paraformaldehyde as described [20]. Brains were sectioned (10-μm thick) coronally and were stained with thionine. The hippocampal CA_1 neurons/mm were counted as described [20,23].

Data were presented as mean ± SD, and analyzed using ANOVA followed by Dunnett's multigroup comparisons post-test (GraphPad Prism Software, San Diego, CA). A value of $P < 0.05$ was considered significant.

Our previous studies showed significant ArAc release immediately after 10-min ischemia (0-min reperfusion) which returned to sham levels by 30-min reperfusion [23]. A later release of ArAc occurred after 1-day [20,21,23]. Treatment with MCPG, MPEP or AIDA 30-min prior to ischemia did not significantly alter the ArAc levels following 10-min ischemia with no reperfusion (data not shown). Our studies then focused on the effects of these agents on ArAc release at 1-day reperfusion. Treatment with MCPG or MPEP significantly ($P < 0.01$ compared to respective vehicle-treated ischemic gerbils, Fig. 1) reduced ArAc levels after 1-day reperfusion, whereas AIDA was not effective (not significant compared to vchl. 1-treated ischemic group, Fig. 1). Similar changes were seen in other FFA including palmitic (16:0), stearic (18:0); and oleic (18:1) (data not shown). DAG levels were not altered after ischemia/1-day reperfusion (vehicles, 165 ± 60 nmol/g tissue) or treatment (166 ± 28) with these agents compared to shams (165 ± 28).

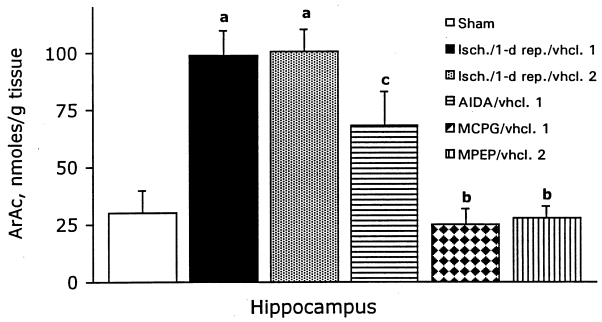

Fig. 1. Effects of MCPG, MPEP and AIDA on ArAc levels in FFA after 10-min forebrain ischemia and 1-day reperfusion in gerbils ($n = 7$ per group). Vhcl. 1 = 0.1 N NaOH adjusted to pH 7.5 for MCPG and AIDA; Vhcl. 2 = 5% DMSO for MPEP. [a] $P < 0.01$ compared to sham; [b] $P < 0.01$ compared to vehicle-treated ischemic group and not significant compared to sham. [c] $P < 0.05$ compared to sham and not significant compared to vehicle-treated ischemic group.

Ischemia resulted in significant neuronal death in the hippocampal CA_1 subfield after 6-day reperfusion (Fig. 2; $n = 7$ per group; neuronal counts: shams 275 ± 10 neurons/

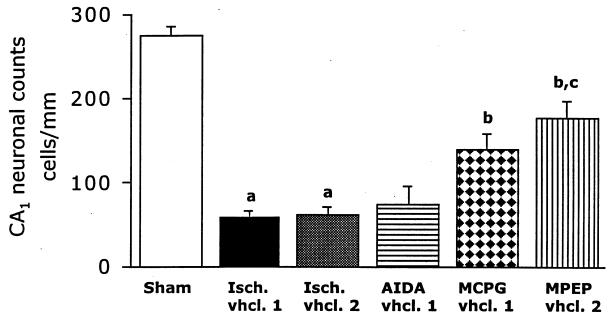

Fig. 2. CA_1 hippocampal neuronal counts after 10-min forebrain ischemia and 6-day reperfusion in gerbils ($n = 7$ per group). Vhcl. 1 and Vhcl. 2 are explained in Fig. 1 legend. [a] $P < 0.01$ compared to sham; [b] $P < 0.01$ compared to vehicle-treated ischemic and compared to shams; [c] $P < 0.05$ compared to ischemia + MCPG.

mm compared to ischemia vhcl. 1: 58 ± 8; vhcl. 2: 62 ± 9; $P < 0.01$ ischemia/vehicles vs. shams). MCPG and MPEP significantly increased ($P < 0.01$) neuronal survival (Fig. 2; $n = 7$ per group; neuronal counts: 140 ± 18 and 177 ± 20 neurons/mm, respectively, $P < 0.01$ compared to respective vehicle-treated ischemic and compared to shams). AIDA did not provide any significant neuroprotection (Fig. 2). Shams treated with mGluR antagonists showed no effect either on ArAc levels or CA_1 neuronal counts (data not shown) which is expected if the receptors are not stimulated in non-ischemic animals.

PLC and phospholipase A_2 (PLA_2) are activated in ischemia by stimulation of group I mGluR, and increases in $[Ca^{2+}]_i$ through NMDA-receptors [10]. PLC cleaves phosphatidylinositides to inositol phosphates and an intermediate DAG, which is further hydrolyzed to FFA including ArAc. PLA_2 cleaves ArAc at the sn-2 position of phosphatidylcholine and phosphatidylethanolamine. Neurotoxicity of the released ArAc is mediated by reactive oxygen species (ROS) generated through its metabolism by cyclooxygenases/lipoxygenases [10,21–23]. Lipid peroxides formed by ROS produce pro-apoptotic aldehydes, including 4-hydroxynonenal and acrolein [2]. ArAc may also stimulate sphingomyelinase to generate pro-apoptotic ceramide [9,20–22].

A release of ArAc was observed after 1-day reperfusion ($P < 0.01$ compared to sham) indicating activation of PLC and/or PLA_2 [20–23]. The fact that MCPG and MPEP attenuated ArAc levels at 1-day suggests the involvement of PLC. Activities of PLC and PLA_2 may be inter-linked, since ArAc, its metabolites, and ROS reduce glutamate uptake processes [10,21,22]. The ArAc metabolite, prostaglandin E_2 stimulates the release of glutamate from astrocytes [1], which in turn activates PLC and PLA_2. Thus, changes in activity of either PLC or PLA_2 may affect the other pathway through this feedback mechanism [10,12,22].

We have previously shown that after 10-min transient ischemia, significant CA_1 neuronal death began at 3-days and continued until 6-days reperfusion [24]. Our results indicate that MCPG and MPEP provided neuroprotection in the hippocampal CA_1 region (Fig. 2). These results are in agreement with other studies that MCPG provided neuroprotection in the CA_1 region after hypoxic injury [25]. MCPG also completely blocked the glutamate-stimulated phosphatidylinositol hydrolysis and showed neuroprotection in CNS trauma [14]. MCPG (mGluR 1 and 5 antagonist, Fig. 2) and MPEP (mGluR 5 antagonist, Fig. 2) [5] showed neuroprotection whereas no significant effect was obtained with AIDA (mGluR 1 antagonist, Fig. 2). These findings are consistent with other studies suggesting that mGluR 5 might be more efficiently linked than mGluR 1 to G-protein coupled PLC [3] and that mGluR 5 is expressed more than mGluR 1 in CA_1 pyramidal neurons [13].

There are multiple pathways involved in ischemic injury [20–23] and ArAc release significantly but not entirely determines the neurological outcome. MCPG and MPEP attenuated ArAc to the same extent but did not provide the same degree of neuroprotection. MCPG is non-specific and is also an antagonist for group II mGluR (negatively coupled to adenylyl cyclase). Since agonists for group II mGluR have shown neuroprotection [15], the beneficial effects of mGluR 5 antagonism by MCPG may be partially offset by its antagonist action on group II mGluR. This could account for the better neuroprotection by MPEP compared to MCPG (Fig. 2). MPEP has greater specificity for mGluR 5, with no effect on NMDA-, AMPA-, kainate receptors or mGluRs 1b, 2, 3, 4a, 7b, 8a [5]. While the specificity of these antagonists for various glutamate receptors has been examined, potential effects on other systems of ArAc metabolism such as cyclooxygenases/lipoxygenases cannot be excluded.

The additional possible mechanism of neuroprotection by mGluR 5 antagonist may be mediated through protein kinase C. DAG is an essential co-factor for protein kinase C activation, which has been shown to have neurotoxic effects [7,27]. Since our results did not show any changes in DAG levels after ischemia/1-day reperfusion or treatment with these agents compared to shams, this mechanism may not have a significant role in these studies.

Several mechanisms may be responsible for the signal induced formation of DAG from: (1) phosphoinositide specific-PLC, (2) phosphatidylcholine (PtdCho) specific-PLC, and (3) phospholipase D action on PtdCho followed by dephosphorylation of phosphatidic acid [8]. In cerebral ischemia the accumulation of cytidine 5′-monophosphate resulting from ATP depletion increases the conversion of PtdCho to DAG [20–22]. The fact that DAG levels were not altered following ischemia or treatment with mGluR antagonists suggests that DAG levels are tightly regulated [11]. PLC hydrolysis of phospholipids releases FFA, in which DAG is only an intermediate [20–23]. Activities of DAG lipases (which hydrolyze DAG to FFA [10]) and DAG kinase (which phosphorylates DAG to phosphatidic acid [8]) appear to be mainly controlled by substrate (DAG) concentration [8]. Thus, as DAG is released from phospholipids, these enzymes may be stimulated to maintain the DAG levels.

In other studies, AIDA showed neuroprotection following 5-min transient ischemia [16] which may be attributable to differences in the duration of ischemia (present study: 10-min transient ischemia). In conclusion we have demonstrated the neuroprotective nature of the mGluR 5 antagonist in vivo. Further studies are needed to determine the role of mGluR in the development of excitotoxic damage that may ultimately lead to novel therapeutic targets in the treatment of CNS injury.

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