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Short communication

The effect of 2-amino-3-phosphonopropionic acid (AP-3) in the gerbil model of cerebral ischaemia

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Abstract

The effect of 2-amino-3-phosphonopropionate (AP-3), a metabotropic glutamate receptor antagonist on behavioural and histological changes following global ischaemia was investigated on the Mongolian gerbil. Ischaemia was induced by bilateral carotid occlusion for 5 min. AP-3 was administered i.p. (25 or 250 mg/kg) 30 min before and 24 h after surgery. Significant neuroprotection was observed 96 h after surgery to cells in the CA1 region of the hippocampus in drug treated animals. AP-3 (250 mg/kg) significantly attenuated the increase in locomotor activity measured 72 h after surgery. These results suggest that metabotropic glutamate receptors play a role in the neurodegeneration seen following ischaemia.

Keywords: Metabotropic glutamate receptor; AP-3 (2-amino-3-phosphonopropionate); Ischemia

1. Introduction

Cerebral ischaemia causes a selective pattern of neurodegeneration (Brierley, 1976). The Mongolian gerbil is a particularly useful animal model of cerebral ischaemia, as it lacks connections between the carotid and vertebrobasilar arteries (Crain et al., 1988). Five minute bilateral carotid occlusion in the gerbil causes uniform destruction of the CA1 pyramidal cells in the hippocampus, whilst the CA3 and CA4 regions are relatively spared (Crain et al., 1988). Although the exact mechanism of ischaemia induced cell death has not been fully elucidated, both in vivo and in vitro experiments have led to several hypotheses on the neurochemical mechanisms involved in ischaemia/hypoxia induced cellular neurodegeneration. There is evidence to suggest that the excitatory amino acid glutamate acts as a neurotoxin in ischaemic/hypoxic cellular damage. Earlier studies showed that the administration of exogenous glutamate caused degeneration of neurons. Glutamate receptors have been divided on the basis of their effector mechanisms. Metabotropic glutamate receptors were first described by the ability of glutamate receptor agonists such as quisqualate and ibotenate to increase phosphoinositide hydrolysis which was distinguished from the effects of ligand-gated ion channel (ionotropic) glutamate receptors, as it was not mimicked by the selective ionotropic glutamate receptor agonists *N*-methyl-D-aspartate (NMDA) and D,L-α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA), or blocked by their respective antagonists (Schoepp et al., 1991). Studies on excitatory amino acid mediated toxicity have suggested that the neurodegenerative mechanism is not restricted to any one receptor type, but that different mechanisms of toxicity are involved (Schurr and Rigor, 1992). Toxicity develops in at least two mechanisms, a rapid degeneration seen within 30 min of the ischaemic incident dependent on calcium influx, and a slow phase involving cellular swelling dependent on intracellular calcium release (Olney et al., 1989). Recent studies have shown that AMPA receptors, and possibly the metabotropic glutamate receptors, evoke the slow mechanism of neurodegeneration. The fast mechanism of glutamate toxicity is directly induced by an excessive influx of calcium ions through NMDA operated calcium channels (Garthwaite and Garthwaite, 1991). These authors suggested that the Purkinje cells and hippocampal neurons were most vulnerable to the delayed and slow mechanisms displayed a 'dark cell degeneration' whose cytological features bore a close

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resemblance to those damaged by ischaemia, hypoglycaemia or status epilepticus in vivo. The metabotropic glutamate receptor (mGlu) agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) has been used to study the possible role of the mGlu's in ischaemic and hypoxic conditions. The results of such studies have been conflicting. Administered with NMDA markedly potentiated the severity of the NMDA-mediated CA1 neuronal damage in the rat (McDonald and Schoepp, 1992). These authors suggested as ACPD alone did not cause neurodegeneration, but that metabotropic glutamate receptors may merely potentiate the damage caused by NMDA. However, others have shown that ACPD attenuated the NMDA neurotoxicity in cortical slices (Koh et al., 1991) and that it was neuroprotective in the rodent model of focal ischaemia (Chiamulera et al., 1992). The aspartate analogue amino-3-phosphonopropionate (AP-3) has provided information on the possible role of the mGlu's in excitatory amino acid induced neurotoxicity. It inhibits [^{3}H]*N*-acetylaspartyl-L-glutamate (NAAG) binding to membranes of rat forebrain, as does the mGlu antagonist 2-amino-4-phosphonobutyrate (L-AP-4) but AP-3 appears to be more selective (Schoepp et al., 1991). Other studies have shown L-AP-3 protects hippocampal neurones from hypoxic damage in vitro (Opitz et al., 1989). The aim of the present study was to investigate the effect of AP-3 on behavioural and histological parameters in the gerbil model of cerebral ischaemia, and to thereby elucidate the possible role of the metabotropic receptors in neuronal degeneration.

2. Materials and methods

Male Mongolian gerbils (at least 3 months old and weighing in excess of 60 g) were obtained from Bantin and Kingman, Hull, UK. The animals were maintained in standard lighting conditions; food and water were freely available. To evaluate the neuroprotective effects of AP-3 (Tocris Cookson, Bristol, UK) six groups of eight gerbils were used. AP-3 was dissolved in saline and administered in a dose of 25 or 250 mg/kg. Saline was administered to the controls. The animals were injected i.p. 30 min and 24 h post-surgery. To perform the bilateral carotid occlusion the animals were anaesthetized with 5% halothane (May & Baker, Dagenham, UK) and anaesthesia maintained using 2% halothane delivered with oxygen at 1 l min^{-1} via a face mask throughout the operation. All animals were maintained at normothermic temperature (37°C) throughout the occluded period and until the animals had recovered from anaesthesia (25–30 min). Core temperatures were continually monitored using a CMA/150 temperature controller/heating pad and brain temperatures were maintained using heating lamps. Through a ventral midline cervical incision, both common carotid arteries were exposed and freed from surrounding connective tissue. In sham operated animals the arteries were freed from surrounding connective tissue but not occluded. In animals to be rendered ischaemic, a short length of Silastic tubing was inserted under each artery. This procedure is known to lead to severe reductions of blood flow in the forebrain (Kato et al., 1989). At the end of the occlusion period (5 min) blood flow was re-established by uncoiling the tubing and removing it. The wound was then sutured and the animals allowed to recover. Locomotor activity was monitored 72 h after surgery for both the control and drug treated animals. Activity was monitored using an automated system, which consisted of four circular Perspex arenas, crossed by six infrared beams. The software allowed for both clockwise and anti-clockwise locomotion. Each animal was monitored for a 30 min period as described by Caldwell et al. (1994). Animals were decapitated on day 4 after the surgery. The brains were removed and fixed in 10% formalin; 5-μm-thick coronal sections at the level of the anterior hippocampus were cut on a Reichert-Jung microtome (Biocut 2035) and stained with haematoxylin-eosin (Yamamuto et al., 1993). The neuronal density in the CA1 region of the hippocampus was measured for all animals using a microscope with built in grid lines. Locomotor and histology counts were analysed using the Mann-Whitney *U*-test. Two-tailed tests of significance were used in evaluating all comparisons, with $P < 0.05$ considered statistically significant. Results are expressed as mean $\pm$ standard error of the mean.

3. Results

Locomotor activity was measured 72 h following surgery, a hyperactive response was observed over the 30 min period ($P < 0.01$) in 5 min bilateral carotid occlusion animals. The results show that while sham operated animals habituated to the arena, the 5 min bilateral carotid occlusion animals remained hyperactive. Post-treatment with AP-3 (25 mg/kg) had no significant effect on the hyperactivity caused by 5 min bilateral carotid occlusion, although it significantly reduced the total activity in the sham operated animals compared to the control animals ($P < 0.05$). The administration of 250 mg/kg AP-3 significantly attenuated the hyperactivity of the bilateral carotid occlusion animals over each time point ($P = 0.021$–0.005). Although 250 mg/kg AP-3 decreased the activity seen in the sham operated animals it did not reach significance (Fig. 1). 5 min bilateral carotid occlusion caused severe neuronal death in the CA1 region of the hippocampus 4 days after surgery which was significantly reversed by

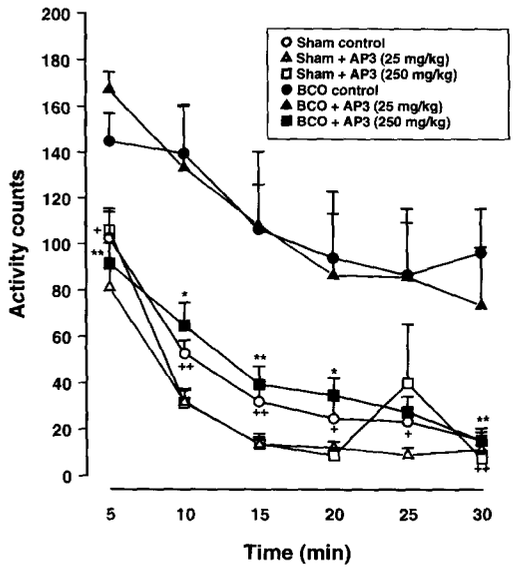

Fig. 1. The effect of AP-3 (25 or 250 mg/kg i.p.) administered 30 min, 24 h after 5 min bilateral carotid artery occlusion (BCO) in male Mongolian gerbils on locomotor activity (72 h post-surgery). * $P < 0.05$ 5 min bilateral carotid occlusion + 250 mg/kg AP-3 treated animals versus 5 min bilateral carotid occlusion control animals. ** $P < 0.001$ 5 min bilateral carotid occlusion + 250 mg/kg AP-3 treated animals versus 5 min bilateral carotid occlusion control animals. + $P < 0.05$ 5 min bilateral carotid occlusion control animals versus sham control. ++ $P < 0.001$ 5 min bilateral carotid occlusion control animals versus sham control.

treatment with both doses of AP-3 (Table 1). Despite the severe hippocampal damage, all animals recovered quickly from surgery and no adverse effects were observed over the 4 days post-occlusion. Only the CA1 pyramidal hippocampal neurons were found to have degenerated; this neurodegeneration was significantly attenuated at both doses of AP-3. There was no significant difference between the sham drug treated animals and the control animals.

4. Discussion

The present study indicates that 5 min bilateral occlusion in the gerbil causes an increase in locomotor activity as measured in a novel environment 72 h after surgery. While the sham operated animals habituated to the test rapidly 5 min bilateral carotid occlusion followed by reperfusion caused a selective pattern of neurodegeneration in the CA1 pyramidal neurons in the hippocampus. It has been hypothesized that such hippocampal damage impairs the ability to produce spatial maps (Chandler et al., 1985). The decreased locomotor activity seen in the bilateral carotid occlusion group following 250 mg/kg AP-3 indicates that there was a significant level of neuroprotection when the metabotropic glutamate receptors are blocked following bilateral carotid occlusion. While AP-3 had a sedative effect on the sham operated animals, this effect was not dose dependent. Previous experiments have shown that AP-3 increases phosphoinositol hydrolysis in hippocampal slices (Desai et al., 1992). In the present study it is assumed that the metabotropic receptors were totally blocked as previously it has been shown that doses of AP-3 250 mg/kg i.p. caused a complete inhibition of phosphoinositol hydrolysis 5 days following drug administration in the hemisected spinal cord preparation (Schoepp et al., 1991). However, in the present experiment AMPA receptor activity was not measured, so that some of the neuroprotection seen in the administration of AP-3 may due to AMPA receptor antagonism. It would appear that AP-3 was neuroprotective in a dose dependent manner; in the low dose AP-3 had no effect on locomotor activity, but was neuroprotective whereas at higher doses it was both neuroprotective and reduced the locomotor hyperactivity. The neuroprotection seen by administering the metabotropic glutamate receptor antagonist AP-3 suggests a link between the different glutamate receptors; such a link may exist in the mechanism of long term potentiation and may also occur in neurodegenerative mechanism observed in cerebral ischaemia. While the precise mechanism is unclear, it is possible the metabotropic receptors together with the AMPA receptors may prime the NMDA receptor sites and by possibly increasing the level of intracellular calcium. This would explain how the metabotropic glutamate receptor agonist ACPD administered with NMDA increased neuronal degeneration, while it had no effect on the AMPA induced damage (McDonald and

Table 1
The effects of AP-3 administered 30 min, and 24 h after 5 min bilateral carotid occlusion (BCO) on the neuronal density in the CA1 layer of the hippocampus 96 h after surgery in the Mongolian gerbil (means ± S.E.M.; $n = 8$)

	Sham control	Sham + 25 mg/kg AP-3	Sham + 250 mg/kg AP-3	5 min BCO	5 min BCO + 25 mg/kg AP-3	5 min BCO + 250 mg/kg AP-3
Neuronal density per 1 mm CA1	177.8 ± 14.3	161.7 ± 4.0	174.2 ± 3.0	40.0 ± 7.1 [a]	61.3 ± 4.6 [b]	178.8 ± 3.6 [c]

[a] $P < 0.001$ 5 min bilateral carotid occlusion control versus sham control. [b] $P < 0.001$ 5 min bilateral carotid occlusion + 25 mg/kg AP-3 versus sham + AP-3 25 mg/kg, $P < 0.05$ 5 min bilateral carotid occlusion + 25 mg/kg AP-3 versus 5 min bilateral carotid occlusion control. [c] $P < 0.001$ 5 min bilateral carotid occlusion + 250 mg/kg AP-3 versus 5 min bilateral carotid occlusion control. For each animal counts were taken in triplicate along the CA1 layer.

Schoepp, 1992). Other studies suggesting increased neuronal degeneration following ACPD administration (Koh et al., 1991) is mediated by increased phosphoinositol hydrolysis could be misleading (Desai et al., 1992). As there is evidence to suggest that ACPD induced excitation of CA1 pyramidal cells, and reductions of excitatory post-synaptic potentials are mediated by mGlu's that are distinct from the AP-3 sensitive phosphoinositol hydrolysis linked receptor (Desai et al., 1992). Clearly there is a need to develop more selective metabotropic glutamate antagonists which may further elucidate the mechanism of neurodegeneration and also provide a novel therapy for the treatment of cerebral ischaemia, without the side effects that may be seen with ionotropic glutamate receptor antagonists.

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