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NMDA receptor antagonism, but not AMPA receptor antagonism attenuates induced ischaemic tolerance in the gerbil hippocampus

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Abstract

Recent studies have shown that a brief 'pre-conditioning' ischaemic insult reduces the hippocampal cell death caused by a subsequent more severe test insult. In the present studies, we have examined the effects of the non-competitive NMDA receptor antagonist ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine, MK-801) a competitive NMDA receptor antagonist, LY202157, AMPA receptor antagonist ((3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2)*H*-tetrazole-5-yl)]decahydroisoquinoline-3-carboxylic acid, LY293558), a non-competitive AMPA receptor antagonist ((−)-1-(4-amino-phenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-3-acetyl-2,3-benzodiazepine, LY300164), and a mixed NMDA/AMPA receptor antagonist, LY246492, in a gerbil model of ischaemic tolerance. Ischaemic tolerance was induced by subjecting gerbils to a 2-min 'pre-conditioning' ischaemia (bilateral carotid occlusion) 2 days prior to a 3-min test ischaemia. The effects of MK-801 (2 mg/kg i.p.), LY293558 (20 mg/kg i.p., followed by 4 × 10 mg/kg at 3 h intervals), LY300164 (4 × 10 mg/kg i.p. at 1 h intervals), LY246492 (40 mg/kg i.p., followed by 4 × 20 mg/kg i.p. at 3 h intervals) and LY202157 (30 mg/kg i.p., followed by 4 × 15 mg/kg i.p. at 2 h intervals) were then examined in this model. Initial dosing commenced 30 min prior to the 2-min 'pre-conditioning' ischaemia. Results indicated that a 2-min 'pre-conditioning' ischaemia produced ischaemic tolerance in all cases. The non-competitive NMDA receptor antagonist, MK-801, produced a significant ($P < 0.01$) reduction in the induced tolerance, while the competitive NMDA receptor antagonist, LY202157, also attenuated ($P < 0.05$) the induction of tolerance. In contrast, two AMPA receptor antagonists (LY293558 and LY300164) and a mixed NMDA/AMPA receptor antagonist (LY246492) had no effect on the induction of tolerance. These results suggest that NMDA receptor activation, but not AMPA receptor activation is involved in the phenomenon of ischaemic tolerance.

Keywords: Ischaemic tolerance; Hippocampus gerbil; AMPA receptor; NMDA receptor; MK-801; LY293558; Neuroprotection

1. Introduction

Transient global cerebral ischaemia in gerbils produces a selective pattern of neuronal damage (Kirino, 1982; Kirino and Sano, 1984; Crain et al., 1988). Five minutes of bilateral carotid artery occlusion produces severe damage in the CA1 pyramidal cell layer of the hippocampus (Kirino and Sano, 1984). The damage in the CA1 pyramidal cells develops slowly, starting 2 days after occlusion, with almost total destruction of the cells being observed 4 days post-occlusion. This phenomenon has been termed 'delayed neuronal death' (Kirino, 1982). The exact mechanisms of damage remain to be fully elucidated, but several mechanisms (activation of voltage-gated calcium channels, excitotoxicity, free radicals, mitochondria and apoptosis) appear to be involved (Boxer and Bigge, 1997; Del Zoppo et al., 1997). The excessive increase of glutamate in the synaptic cleft following ischaemia is thought to play a critical role in the development of neuronal damage (Butcher et al., 1990). Several studies have indicated that many compounds acting at excitatory amino acid receptors have beneficial effects against cerebral ischaemia (Park et al., 1988, 1992; Bullock et al., 1990, 1994; Sheardown et al., 1990). Many of the early studies demonstrated that NMDA receptor antagonists are neuroprotective in animal models of global and focal cerebral ischaemia (Simon et al., 1984; Park et al., 1988, 1992; McCulloch, 1992). Other studies have focused on the neuroprotective actions of AMPA receptor antagonists in animal models of global (Sheardown et al., 1990, 1993; Lodge et al., 1996; O'Neill et al., 1998) and focal (Bullock et al., 1994; Gill, 1994; Gill and Lodge, 1995; Gill et al., 1992; Graham et al.,

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1996; Yatsugi et al., 1996; Shimizu-Sasamata et al., 1998) cerebral ischaemia.

In 1991, Kirino et al. reported that a 2-min 'pre-conditioning' occlusion, 2 days prior to a 5-min occlusion, led to a significant reduction in hippocampal cell loss compared with animals subjected to 5-min ischaemia alone. The authors also reported that this brief ischaemia caused an increased in 70 kDa heat shock protein and that this may render the neurones more tolerant to subsequent metabolic stress (Kirino et al., 1991). More recent studies have indicated that this tolerance is lost if the second ischaemia is carried out 4 weeks after the first brief ischaemia (Chen et al., 1994). However, if a second 'pre-conditioning' ischaemia is carried out before the second 5-min ischaemia, at 4 weeks, the neurones are protected indicating that ischaemic tolerance can be induced repeatedly in gerbil hippocampal neurones (Chen et al., 1994). Other studies have shown that transient forebrain ischaemia also protects against subsequent focal cerebral ischaemia in rats (Matsushima and Hakim, 1994).

The mechanism of this induced tolerance is not clear, but brief periods of ischaemia alter gene expression and protein synthesis. Several studies have indicated an increase in heat shock proteins (Kirino et al., 1991) and other studies have shown regional increases in apoptotic gene expression (Chen et al., 1996). Further studies have reported that (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) attenuates the production of HSP-72 in the CA1 neurones and inhibits the induction of tolerance to ischaemia in the gerbil (Kato et al., 1992). However, the same study reported that anisomysin (a protein synthesis inhibitor) reduced HSP-72 synthesis, but failed to inhibit the induction of tolerance.

We wanted to further evaluate the role of NMDA receptor blockade on induced ischaemic tolerance and investigate if AMPA receptor antagonists, which provide greater protection in global ischaemia, also inhibited the induction of tolerance at neuroprotective doses. Therefore, in the present studies, we have examined the effects of a non-competitive NMDA receptor antagonist (MK-801), a competitive NMDA receptor antagonist (LY202157), a non-competitive AMPA receptor antagonist ((−)-1-(4-amino-phenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-3-acetyl-2,3-benzodiazepine, LY300164), a competitive AMPA receptor antagonist ((3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2)*H*-tetrazole-5-yl)]decahydroisoquinoline-3-carboxylic acid, LY293558) and a mixed NMDA/AMPA rece-t6r antagonist (LY246492) in a gerbil model of ischaemic tolerance.

2. Materials and methods

2.1. Animals and surgery

Male Mongolian gerbils (Bantin and Kingman), weighing in excess of 60 g were used. The animals were maintained in standard lighting conditions and food and water were available ad libitum. The animals were anaesthetised with a 5% halothane/oxygen mixture and maintained using 2% halothane delivered with oxygen at 2 l/min via a face mask throughout the operation. Through a midline cervical incision, both common carotid arteries were exposed and freed from surrounding connective tissue. In animals to be rendered ischaemic, both common carotid arteries were clamped for 2 min. At the end of the occlusion period, blood flow was re-established. In sham operated animals, the arteries were exposed but not occluded. The wound was then sutured and the animals allowed to recover. Throughout surgery body temperature was maintained at 37°C using a temperature controller/heating pad. After surgery the animals were placed in a four compartment thermacage (Beta Medical and Scientific, UK) which maintained the environmental temperature at 28°C. Two days following the 2-min 'pre-conditioning' occlusion or sham-operation, animals were re-anaesthetised and subjected to a 3-min occlusion under the same conditions as above.

2.2. Histological examination

Five days later, the animals were re-anaesthetised and perfused transcardially with 30 ml of 0.9% saline followed by 100 ml of 10% buffered formalin solution. The brains

Table 1
Illustrates neuroprotective effects AMPA and NMDA receptor antagonists in a gerbil model of global ischaemia
Compound dosing commenced 30 min prior to a 5-min period of bilateral carotid occlusion. Results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampus and as mean ± S.E.M. Percent neuroprotection compared to 5 min occluded control animals. Data are based on groups of 8–10 animals.

Compound	Action	Dose (i.p.)	No. viable cells/mm CA1 hippocampus	% Neuroprotection
Ischaemic control	–	–	11 ± 2	–
MK-801	NMDA antagonist	2 mg/kg	73 ± 8	34 ± 12
LY202157	NMDA antagonist	40 + 4 × 20 mg/kg	229 ± 15	99 ± 13
		30 + 4 × 15 mg/kg	50 ± 25	24 ± 7
LY300164	AMPA antagonist	4 × 10 mg/kg	35 ± 12	24 ± 2
LY293558	AMPA antagonist	20 + 4 × 10 mg/kg	132 ± 29	56 ± 14
LY246492	Mixed AMPA/NMDA antagonist	40 + 4 × 20 mg/kg	86 ± 22	41 ± 16

were removed and placed in 10% formalin for 3 days, processed and embedded in paraffin wax. 5 mm coronal sections were taken 1.5, 1.7 and 1.9 mm caudal to the bregma in the anterior hippocampus using a sledge (Leitz 1400) microtome. The slices were stained with haematoxylin and eosin and the neuronal density in the CA1 subfield of the hippocampus was measured using a microscope with grid lines (0.05 mm × 0.05 mm). The neuronal density was expressed as the number of viable cells per mm CA1 hippocampus. Statistical analysis of histological data was assessed using ANOVA followed by Student's *t*-test with Bonferroni corrections and *P*-values < 0.05 were considered statistically significant.

2.3. Drug administration

LY293558 (20 mg/kg i.p., followed by 4 × 10 mg/kg i.p. at 3 h intervals), LY300164 (10 mg/kg i.p. × 4 at 1 h intervals), LY202157 (30 mg/kg i.p., followed by four doses of 15 mg/kg i.p. at 2 h intervals), LY246492 (40

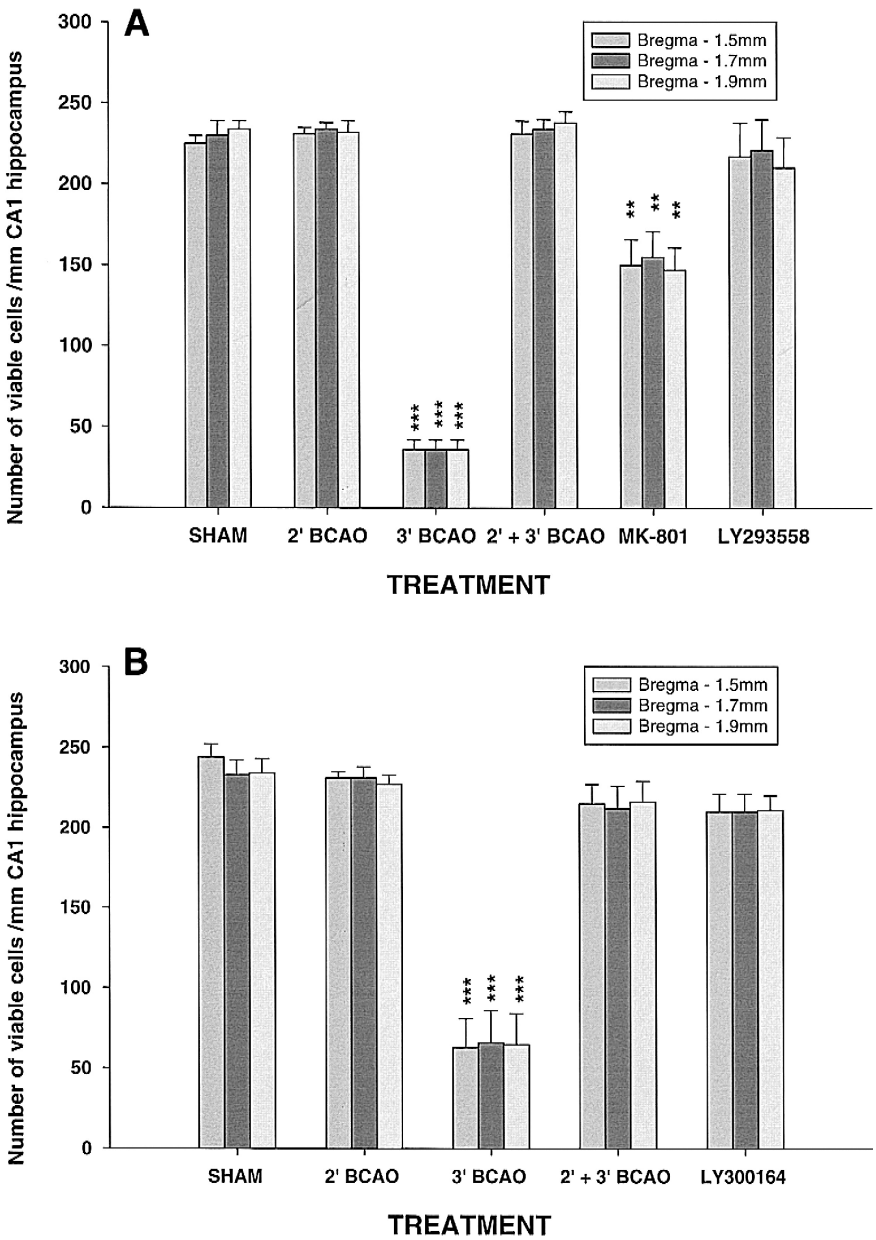

Fig. 1. (A and B) Effect of MK-801, LY293558 and LY300164 on induced ischaemic tolerance in the gerbil hippocampus (stereotaxic level 1.7 mm caudal to bregma). The bars from left to right represent mean ± S.E.M. ($n = 8$) of the number of viable CA1 pyramidal cells following (i) sham occlusion, (ii) a 'pre-conditioning' 2-min ischaemia alone, (iii) a 3-min test ischaemia alone, (iv) a 2-min ischaemia followed 2 days later by a 3-min ischaemia (tolerance), (v) and (vi) drug pretreatment prior to the 2-min 'pre-conditioning' ischaemia, followed 2 days later by a 3-min ischaemia (no drugs). MK-801 was dosed at 2 mg/kg i.p. and produced a significant reduction ($P < 0.01$) in the induced tolerance (A). LY293558 at 20 mg/kg i.p., followed by four doses of 10 mg/kg i.p. (A) and LY300164 at four doses of 10 mg/kg i.p. (B) had no significant effect on the induced tolerance.

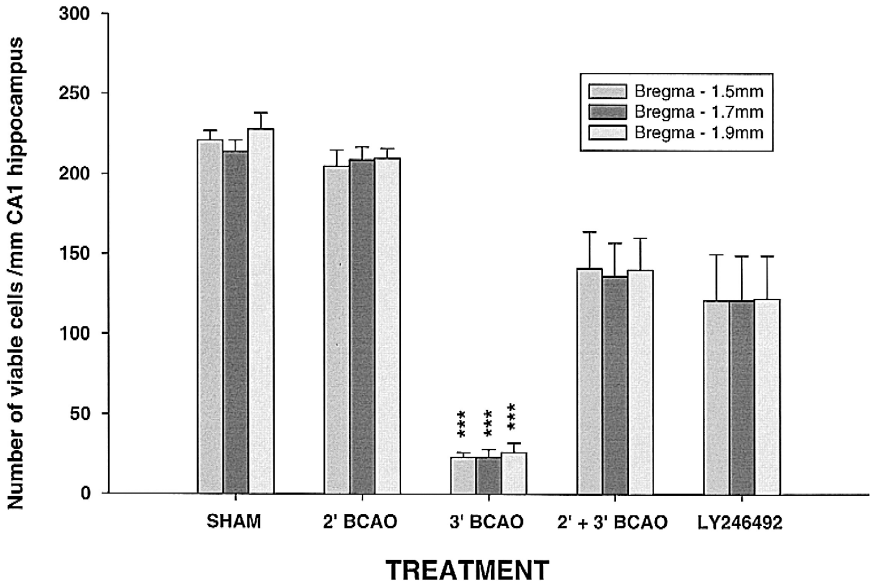

Fig. 2. Effect of LY246492 on induced ischaemic tolerance in the gerbil hippocampus (stereotaxic level 1.7 mm caudal to bregma). The bars from left to right represent mean ± S.E.M. ($n = 10$) of the number of viable CA1 pyramidal cells following (i) sham occlusion, (ii) a 'pre-conditioning' 2-min ischaemia alone, (iii) a 3-min test ischaemia alone, (iv) a 2-min ischaemia followed 2 days later by a 3-min ischaemia (tolerance), and (v) drug pretreatment prior to the 2 min 'pre-conditioning' ischaemia, followed 2 days later by a 3-min ischaemia (no drugs). LY246492 was dosed at 40 mg/kg i.p., followed by four doses of 20 mg/kg i.p. and had no significant effect on the induced tolerance.

mg/kg i.p., followed by four doses of 20 mg/kg i.p. at 3 h intervals) and MK-801 (2 mg/kg i.p.) were administered 30 min before the 2-min 'pre-conditioning' ischaemia. The above doses were chosen as being 'equi-neuroprotective', i.e., in previous experiments with a severe ischaemic insult (5 min occlusion) they were each shown to provide 25%–50% neuroprotection (Table 1).

3. Results

In the present study, we observed that gerbils subjected to a sham operation, followed 2 days later by a 3-min test occlusion, had significant damage in the CA1 region of the hippocampus (Fig. 1a,b). This damage was abolished if the 3-min occlusion was preceded by a 2-min 'pre-conditioning' occlusion instead of a sham operation (Fig. 1a,b). Administration of the ampa receptor antagonists LY293558 (20 mg/kg i.p., followed by 4×10 mg/kg i.p. at 3 h intervals) or LY300164 (4×10 mg/kg i.p. at 1 h intervals) had no effect on the induced tolerance (Fig. 1a,b). In contrast, pretreatment with the non-competitive nmda receptor antagonist, MK-801, at a single dose of 2 mg/kg i.p., produced a significant reduction ($P < 0.001$) in the induced tolerance (Fig. 1a).

Administration of the mixed AMPA/NMDA receptor antagonist, LY246492 (40 mg/kg i.p., followed by 4×20 mg/kg i.p. at 3 h intervals), had no effect on the induced tolerance (Fig. 2).

Similarly, administration of the competitive NMDA receptor antagonist, LY202157 (30 mg/kg i.p., followed by 4×15 mg/kg i.p. at 2 h intervals), had no effect on the induction of ischaemic tolerance (Fig. 3a). However, when administered at 40 mg/kg i.p., followed by 4×20 mg/kg at 2 h intervals LY202157 caused some attenuation the induction of tolerance (Fig. 3b).

In all experiments, no significant difference in cell numbers was observed between the three stereotaxic levels in each experimental group.

4. Discussion

The pyramidal cells of the CA1 and CA2 regions of the hippocampus are exceptionally vulnerable to periods of global ischaemia in both animals and man (Brierley and Graham, 1984). The Mongolian gerbil is used as a model of global cerebral ischaemia due to the fact that the animal has a unique cerebral circulation, lacking connections between the carotid and vertebro-basilar circulations (Levy et al., 1975). Consequently, bilateral occlusion of the common carotid arteries results in a near complete forebrain ischaemia and delayed damage to the CA1 region of the hippocampus. Increases in extracellular glutamate in ischaemia result in the activation of NMDA and non-NMDA receptors. NMDA receptor activation results in the influx of sodium and Ca^{2+} ions into the cell, while AMPA receptor activation allows sodium, and in some cases Ca^{2+}, entry into the cell (Boxer and Bigge, 1997). The resulting depolarisation exacerbates Ca^{2+} entry through NMDA receptors and voltage-gated calcium channels. This increase in intracellular Ca^{2+}, along with the disruption of

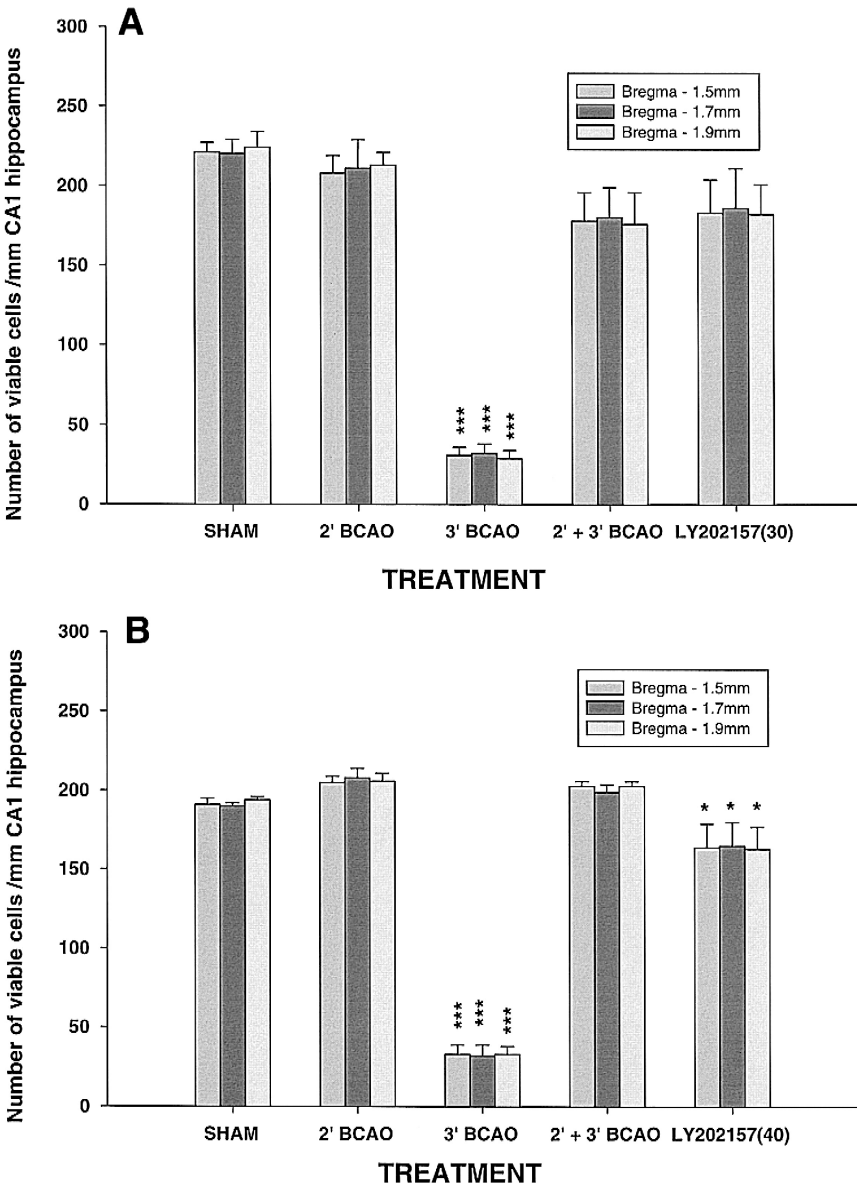

Fig. 3. (A and B) Effect of LY202157 on induced ischaemic tolerance in the gerbil hippocampus (stereotaxic level 1.7 mm caudal to bregma). The bars from left to right represent mean ± S.E.M. ($n = 8$) of the number of viable CA1 pyramidal cells following (i) sham occlusion, (ii) a 'pre-conditioning' 2-min ischaemia alone, (iii) a 3-min test ischaemia alone, (iv) a 2-min ischaemia followed 2 days later by a 3-min ischaemia (tolerance), and (v) drug pretreatment prior to the 2-min 'pre-conditioning' ischaemia, followed 2 days later by a 3-min ischaemia (no drugs). LY202157 was dosed at 30 mg/kg i.p., followed by four doses of 15 mg/kg i.p. (A) and had no significant effect on the induced tolerance. At 40 mg/kg i.p., followed by four doses of 20 mg/kg i.p., LY202157 produced a significant attenuation of the induced tolerance ($P < 0.05$) (B).

homeostatic mechanisms for intracellular Ca^{2+}, results in the activation of calcium-dependent catabolic processes leading to cell death (Boxer and Bigge, 1997). Autoradiographic studies have demonstrated that the dendritic layers of the CA1 and CA2 neurones of the rodent hippocampus possess a high density of NMDA and AMPA receptors (Monaghan et al., 1989).

In the present studies, we examined the neuroprotective effects of a non-competitive (MK-801), a competitive (LY202127) NMDA receptor antagonist, a non-competitive AMPA (LY300164) and a competitive AMPA (LY293558) receptor antagonist and found that they protect against the ischaemic induced hippocampal damage. These results are in agreement with studies that have shown that NMDA (Simon et al., 1984; Park et al., 1988, 1992; Bullock et al., 1990; McCulloch, 1992; Hayward et al., 1993; Li and Buchan, 1993; Hicks et al., 1999) and AMPA (Sheardown et al., 1990, 1993; Bullock et al., 1994; Gill, 1994; Gill and Lodge, 1995; Gill et al., 1992; Xue et al., 1994; Graham et al., 1996; Lodge et al., 1996; Yatsugi et al., 1996; Kawasaki-Yatsugi et al., 1998; Shimizu-Sasamata et al., 1998) receptor antagonists are neuroprotective in animal models of cerebral ischaemia. We have also previously reported protective effects with

NMDA (Hicks et al., 1999) and AMPA receptor (Lodge et al., 1996; O'Neill et al., 1998) antagonists in this model. However, the side effect profile of earlier NMDA receptor antagonists (psychomimetic, learning and memory impairment) prevented clinical development of several of these compounds (Morris et al., 1986; Koek et al., 1988). Further studies by Olney indicated that the non-competitive NMDA receptor antagonist (MK-801) produced vacuoles and neuronal damage in the posterior and retrosplenial cortex (Olney et al., 1990, 1991). Further problems (solubility, dose-limiting adverse effects, attaining neuroprotective concentrations, etc.) have plagued clinical trials with NMDA receptor antagonists in ischaemic stroke (Lees, 1997).

We carried out further studies to evaluate the effects of neuroprotective doses of these compounds in a gerbil model of ischaemic tolerance. In the gerbil brain, delayed neuronal death can be reduced by 'pre-conditioning' with a short subthreshold period of ischaemia (Kirino et al., 1991), 2 days prior to a subsequent more damaging insult. In the present studies, we observed good tolerance in all experiments. In the present studies, pre-treatment with the non-competitive NMDA receptor antagonist, MK-801, reduced the induced ischaemic tolerance, suggesting the involvement of NMDA receptor activation. These results are in agreement with studies by Kato et al. (1992) which indicated that MK-801 blocks the induction of tolerance. This effect could be perceived to be detrimental to the brain's natural defence against ischaemic conditions. High doses of the competitive NMDA receptor antagonist, LY202157, also produced some attenuation of the induced tolerance.

As mentioned earlier AMPA receptor antagonists are another potential target for treatment of ischaemic conditions. Initial studies demonstrated that quinoxalinediones, such as 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-[*f*]quinoxaline (NBQX), are neuroprotective in models of cerebral ischaemia (Sheardown et al., 1990; Gill et al., 1992). Further studies indicated that delayed treatment with NBQX was neuroprotective in gerbils (Sheardown et al., 1993) and rats (Li and Buchan, 1993). However, earlier AMPA receptor antagonists (such as NBQX) suffered the additional problems of low solubility and nephrotoxicity (Xue et al., 1994). Newer AMPA receptor antagonists, 6-(1-imidazolyl)-7-nitroquinoxaline-2,3-(1*H*,4*H*)-dione monohydrochloride (YM90K) and the related compound ([2.3-dioxo-7-(1*H*-imidazol-1-yl)6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl] acetic acid monohydrate (YM872)), with better solubility have been described. Both YM90K and YM872 have neuroprotective effects in models of global and focal cerebral ischaemia (Yatsugi et al., 1996; Kawasaki-Yatsugi et al., 1998; Shimizu-Sasamata et al., 1998), but are still based on the quinoxalinedione structure.

Recently, it has been reported that a new series of AMPA receptor antagonists based on (3*SR*,4*aRS*,6*RS*, 8*aRS*)-6-[2-(1*H*-tetrazol-5-yl)-ethyl]-1,2,3,4*a*,5,6,7,8*a*-decahydro-isoquinoline-3-carboxylic acid (LY215490) are neuroprotective in a rat model of focal ischaemia (Gill and Lodge, 1995). It has also been shown that the active isomer of LY215490, which is LY293558 is neuroprotective in a cat model of cerebral ischaemia (Bullock et al., 1994). Further evaluation of these decahydroisoquinolines structures indicated that the series contained compounds such as LY377770, which provided greater neuroprotection than the parent compound (O'Neill et al., 1998). The compounds are soluble and have a good (2 h) time window of protection suggesting these molecules could be useful for the treatment of stroke. Furthermore, in the present, we have demonstrated that the AMPA receptor antagonists based on the decahydroisoquinoline (LY293558) or 2,3-benzodiazepine (LY300164) structures, did not reduce the induced tolerance, suggesting little or no AMPA receptor involvement. This could be looked upon as a favourable effect, i.e., the brain's natural protective mechanisms remain intact. The mixed AMPA/NMDA receptor antagonist, LY246492 did not block the ischaemic tolerance. This may be because (1) in that experiment the tolerance effect was smaller than the other experiments or (2) the compound has both NMDA and AMPA activity and was therefore tolerated at doses that only partially block NMDA receptors. The exact mechanism by which NMDA receptor antagonists block the induction of ischaemic tolerance remains unclear as both NMDA and AMPA receptor antagonists protect against ischaemic brain injury. It is also of interest to note that high doses of MK-801 induce HSP-70 in several brain regions (in particular the posterior cingulate and retrospenial cortex), whereas AMPA antagonists do not. This would suggest that HSP-70 is not involved in the tolerance phenomena as NMDA antagonists would increase rather than block the tolerance. However, it has been suggested that chemical induction of tolerance is possible and further examination of gene and protein expression following pharmacological intervention may help elucidate mechanisms involved and produce chemical or pharmacological methods of inducing tolerance.

Another issue with the protective effects of earlier excitatory amino acids has been the involvement of hypothermia. Several studies have demonstrated that intra-ischaemic hypothermia is neuroprotective (Busto et al., 1987; Barone et al., 1997). Other studies have reported that the delayed hypothermia is also protective in global and focal cerebral ischaemia (Busto et al., 1989; Colbourne and Corbett, 1994) and therefore delayed hypothermia could also have effects on ischaemic tolerance. In contrast other reports indicate that intra-ischaemic, but not post-ischaemia hypothermia is neuroprotective (Dietrich et al., 1993). The discovery that MK-801 caused hypothermia led to the suggestion that MK-801 was providing neuroprotection by producing hypothermia (Buchan and Pulsinelli, 1990; Corbett et al., 1990), while others postulate that the

protective actions of MK-801 are mediated by a small transient hypothermia that acts synergistically with the drug to yield neuroprotection (Hayward et al., 1993). In fact, studies by Green et al. (1995) demonstrated that combined hypothermia and delayed treatment with MK-801 was more effect than either alone in a rat model of global ischaemia. Other recent studies have suggested that the protective effect of NBQX may also be due to hypothermia (Nurse and Corbett, 1996). In the present studies to monitor temperature effects rectal temperatures were measured periodically for 6 h after occlusion and the animals were placed in four compartmental thermacages (which maintained environmental temperatures at 28°C) immediately after surgery and remained there for a minimum of 12 h. We did not monitor brain temperature so a possible contribution of brain temperature changes to the tolerance cannot be totally excluded. In addition, we cannot rule out the possibility of delayed hypothermia contributing to the observed effects, but in all cases the animals were fully recovered when returned to their home cages.

The cellular and molecular mechanisms underlying the tolerance phenomenon are poorly understood, although it has been suggested that processes such as selective gene expression and a subsequent increase in protein synthesis may be involved. Several studies have shown that there is regional induction of immediate early genes such as c-*fos* and c-*jun* following ischaemia (Uemura et al., 1991; Ikeda et al., 1994) and fluid percussion injury (Raghupathi et al., 1995). In addition, stress proteins, such as HSP-70, HSP-72 and HSP-27 are induced following ischaemic episodes (Nowak, 1985; Vass et al., 1988; Kato et al., 1995a,b,c). However, several investigators have reported that there is no correlation between the induction of stress proteins (Kato et al., 1995a; Li et al., 1995) and the manifestation of ischaemic tolerance. Instead, it appears that HSP-70, HSP-72, glial fibrillary acidic protein (GFAP), activated microglia, etc. may be markers of acute neuronal stress in penumbral neurones or markers of neuronal damage (Kato et al., 1995b; Li et al., 1995). Other possible candidates are apoptotic related genes. For example, the apoptosis effector gene, *bax*, is reported to be up-regulated in the CA1 region following global cerebral ischaemia (Chen et al., 1996). In contrast, the anti-apoptotic gene, *bcl*-2, is not expressed in the CA1 region, but is expressed in CA3, a region less susceptible to ischaemia (Chen et al., 1996).

There is also evidence for the role of glutamate in ischaemic tolerance and it has been reported that in vitro exposure of cerebellar granule cells to non-lethal concentrations of NMDA reduces susceptibility to subsequent lethal glutamate challenge (Marini and Paul, 1992). The authors also demonstrated that such tolerance is blocked by NMDA receptor antagonists. In another recent study, it was reported that sublethal exposure of cortical cultures to short periods of oxygen–glucose deprivation induced preconditioning neuroprotection, which was blocked by the NMDA antagonist, 3-((*D*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (Grabb and Choi, 1999). The tolerance observed in the present studies cannot be explained by reduced excitatory amino acid release as it has been shown that levels of excitatory amino acid are the same in tolerant and non-tolerant animals (Nakata et al., 1993). Kasischke et al. (1996) have reported that the NMDA receptor antagonist, D-2-amino-5-phosphonopentanoic (AP5), but not the AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), reduced tolerance in hippocampal slices when administered at the time of the 'pre-conditioning' hypoxia.

In conclusion, in the present studies, we have examined the effects of NMDA receptor antagonists and AMPA receptor antagonists in the gerbil model of ischaemic tolerance. The non-competitive NMDA receptor antagonist, MK-801, significantly blocked the induction of tolerance, while the competitive NMDA receptor antagonist, LY202157 provided a partial block. In contrast, AMPA receptor antagonists failed to block the induction of tolerance. Therefore, these results suggest that NMDA receptor activation, but not AMPA receptor activation plays a role in the induction of ischaemic tolerance. They also suggest that AMPA receptor antagonists may be preferable as drug candidates when repeated ischaemic episodes occur.

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