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Neuroprotective effects of the neuronal Ca^{2+} channel blockers, LY042826 and LY393615 in vivo

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Abstract

In the present studies, we have examined the effects of two new Ca^{2+} channel blockers, LY042826 (*N*-{2-[(2-methylphenyl)(phenyl)methoxy]ethyl}-1-butanamine hydrochloride) and LY393615 (*N*-{[5,5-bis(4-fluorophenyl)tetrahydro-2-furanyl]methyl}-1-butanamine hydrochloride) in the gerbil model of global and the endothelin-1 rat model of focal cerebral ischaemia in vivo. Results indicated that both LY042826 ($P < 0.01$) and LY393615 ($P < 0.001$) provided significant protection against ischaemia-induced hippocampal damage in global cerebral ischaemia when dosed at 15 mg/kg i.p. 30 min before and 2 h 30 min after occlusion. In further studies, LY042826 ($P < 0.05$) and LY393615 ($P < 0.01$) were also protective when administered at 15 mg/kg i.p. immediately after and 3 h post-occlusion. Both compounds also provided a significant reduction in the infarct volume following endothelin-1 middle cerebral artery occlusion in the rat when administered at 15 mg/kg i.p. immediately ($P < 0.05$) after occlusion. This protection was similar to that observed with the NMDA receptor antagonist (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine), MK-801 in this model. In conclusion and as a result of the present studies, we report that the novel Ca^{2+} channel blockers, LY042826 and LY393615 protect against ischaemia-induced brain injury in gerbils and rats. The compounds were neuroprotective when administered post-occlusion and may therefore be useful anti-ischaemic agents.

Keywords: Cerebral ischaemia; Neuroprotection; Ca^{2+} channel, neuronal

1. Introduction

It is well established, that following cerebral ischaemia, there is a large increase in extracellular neurotransmitter levels (Globus et al., 1988). The high levels of these neurotransmitters (in particular, glutamate) are thought to cause excitotoxicity, leading to a rise in intracellular free Ca^{2+}, activation of Ca^{2+} dependent proteases, membrane degeneration, mitochondrial dysfunction, free radical production and ultimately cell death (Siesjö, 1992a,b). Attempts to block the various pathways leading to ischaemic cell death, have been arduously investigated (Boxer and Bigge, 1997; Del Zoppo et al., 1997).

It has been shown that agents which block voltage sensitive Ca^{2+} channels, therefore preventing Ca^{2+} entry and controlling neurotransmitter release, reduce the susceptibility of neurones to ischaemic injury. This evidence therefore provides a promising approach against ischaemia-induced damage. Studies with synthetic conopeptides provided scientists with the opportunity to evaluate the therapeutic potential of selective blockade of Ca^{2+} channels in cerebral ischaemia. These results indicated that a single bolus intravenous administration of ω-conotoxin MVIIA (SNX-111), which blocks N-type Ca^{2+} channels provided protection when administered as late as 24 h after the ischaemic insult (Valentino et al., 1993). Similar effects were observed in two other studies (Smith and Siesjö, 1992; Zhao et al., 1994). Omega-conotoxin MVIIA has also been found to be highly effective in reducing the neocortical infarct volume in rat models of focal ischaemia, both when administered during the occlusion (Takizawa et al., 1995) and after the ischaemic episode (Buchan et al., 1994).

One major drawback of conotoxins is that they are large peptide molecules which does not enable the crossing of the blood brain barrier efficiently. Consequently, they have to be administered at high doses that often yields peripheral side effects. However, more recent studies have described neuroprotective effects with smaller non-peptide

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compounds that inhibit neuronal Ca^{2+} channels. For example, it has been reported that NNC 09-0026 (Sheardown et al., 1993; Barone et al., 1994), SB 201823-A (Benham et al., 1993; Barone et al., 1995) and NS-649 (Varming et al., 1996) have protective actions in animal models of cerebral ischaemia.

At Eli Lilly, a high throughput screen using HEK293 cells (human embryonic kidney cells) transfected with human α1A, α1B and α1E Ca^{2+} channel subunits was employed. These subunits represented N, P/Q and R-type Ca^{2+} channels (Tsien et al., 1995). As a result, compounds were identified that inhibited N, P and Q-type Ca^{2+} channels. LY042826 (10 μM) inhibited α1A, α1B and α1E human Ca^{2+} channel subunits $18 \pm 11\%$, $75 \pm 12\%$, $69 \pm 16\%$ and blocked KCl-induced glutamate release $56 \pm 10\%$. LY393615 was more potent and at 10 μM inhibited α1A, α1B and α1E human Ca^{2+} channel subunits $75 \pm 16\%$, $86 \pm 8\%$, $75 \pm 16\%$ and blocked KCl-induced glutamate release $77 \pm 6\%$ (Thomas et al., 1999). LY393615 was also evaluated on Ca^{2+} currents in HEK 293 cells transfected with human α1B or α1E Ca^{2+} channel subunits and on P-type Ca^{2+} channels in isolated Purkinje cells using voltage patch clamp techniques. LY393165 inhibited peak Ca^{2+} currents dose-dependently with IC_{50} values of 1.9 ± 0.1 μM in α1B cells, 5.2 ± 1.1 μM in α1E cells and 4.0 ± 1.0 μM in Purkinje cells.

This report summarises the neuroprotective effects of LY042826 and LY393615 in gerbil global ischaemia and rat focal cerebral ischaemia in vivo. We also studied the neuroprotective effect of LY042826 and LY393615 in comparison to those of NCC 09-0026, SB201823A, CNS1237 and NS-649 in our global ischaemia model.

2. Materials and methods

2.1. Global ischaemia

Male Mongolian gerbils (Bantin and Kingman, Hull, UK) at least 3 months old and weighing in excess of 60–80 g were used. The animals were maintained in standard lighting conditions, 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 1 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

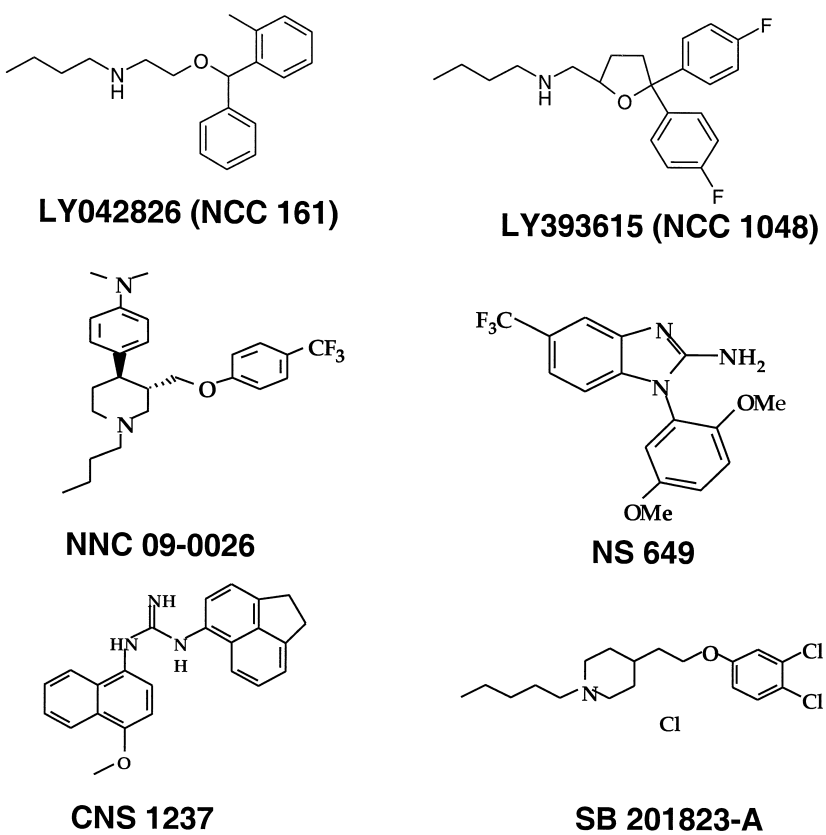

Fig. 1. Illustrates the structure of *N*-{2-[(2-methylphenyl)(phenyl)methoxy]ethyl}-1-butanamine hydrochloride, LY042826); (*N*-{[5,5-bis(4-fluorophenyl)tetrahydro-2-furanyl]methyl}-1-butanamine hydrochloride, LY393615); NNC 09-0026), (2-amino-1-(2,5-dimethoxyphenyl)-5-trifluoromethyl benzimidazole, NS-649); (*N*-acenaphthyl-*N*′-4-methoxynaphth-1-yl guanidine, CNS1237) and (4-[2-(3,4-dichlorophenoxy)ethyl]-1-pentyl piperidine hydrochloride, SB 201823-A).

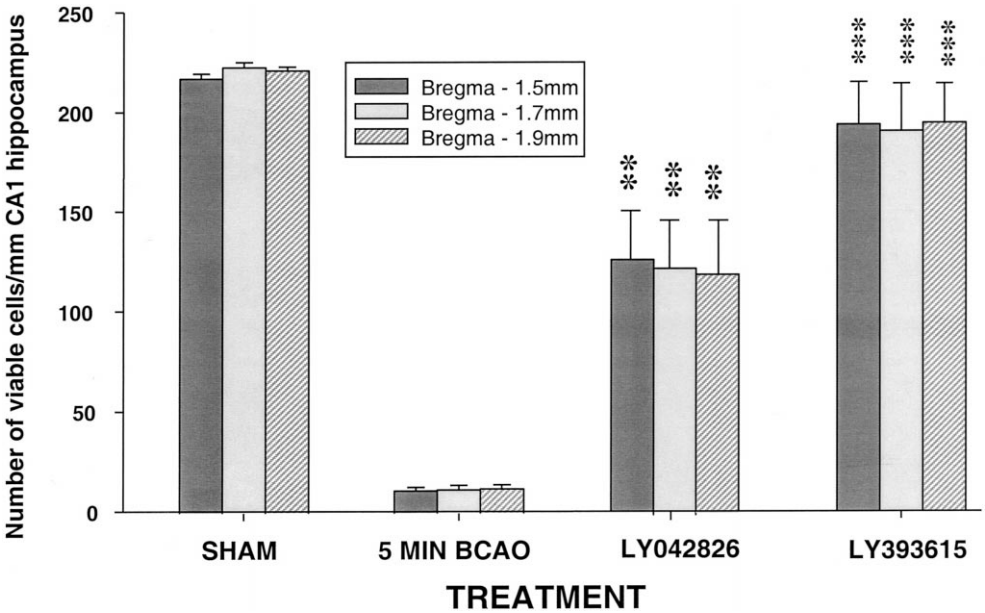

Fig. 2. The effects of LY042826 and LY393615 on the neuronal density in the CA1 region of the hippocampus 5 days after 5 min bilateral carotid artery occlusion. Both compounds were administered at 15 mg/kg i.p. 30 min pre- and 2 h 30 min post-occlusion. Results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampus ($n = 8$ animals per group). The 5-min bilateral carotid artery occlusion caused a severe loss in neuronal cells in the CA1 region ($P < 0.001$). Both LY042826 and LY393615 provided significant neuroprotection against the ischaemia-induced cell death. $^{**}P < 0.01$ vs. 5 min bilateral carotid artery occlusion control, $^{***}P < 0.001$ vs. 5 min bilateral carotid artery occlusion control. Student's *t*-test.

rendered ischaemic, both common carotid arteries were clamped for 5 min. At the end of the occlusion period, blood flow was reestablished. In sham-operated animals, the arteries were exposed but not occluded. The wound was then sutured and the animals allowed to recover. Throughout the surgical procedure, body temperature was maintained at 37°C using a "K-TEMP" temperature controller/heating pad (International Market Supply, Cheshire,

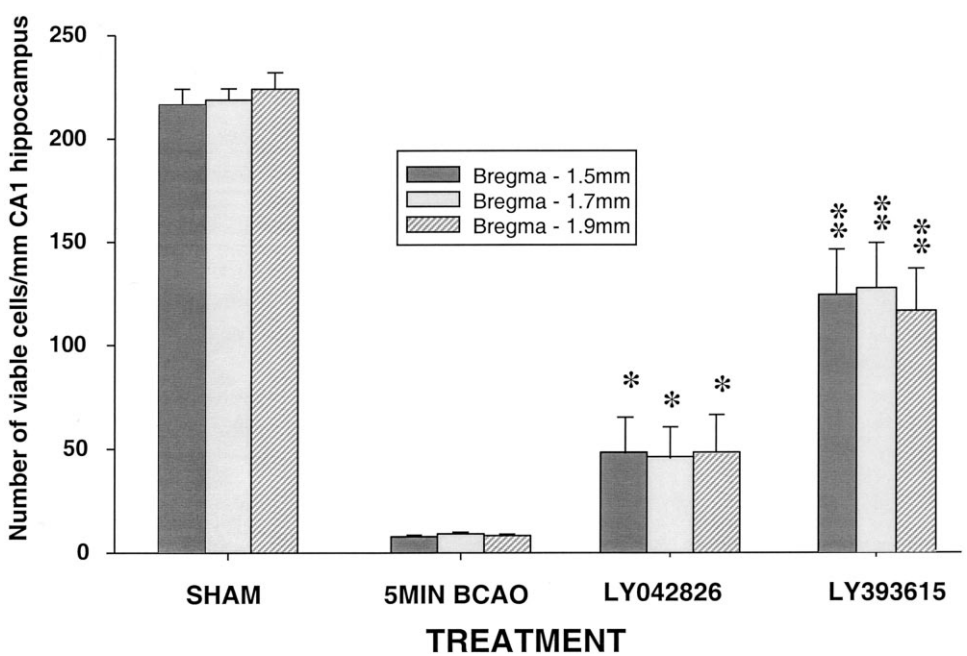

Fig. 3. The effects of LY042826 and LY393615 on the neuronal density in the CA1 region of the hippocampus 5 days after 5 min bilateral carotid artery occlusion. Both compounds were administered at 15 mg/kg i.p. immediately and 3 h post-occlusion. Results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampus ($n = 8$ animals per group). The 5-min BCAO caused a severe loss in neuronal cells in the CA1 region ($P < 0.001$). Both LY042826 and LY393615 provided significant neuroprotection against the ischaemia-induced cell death. $^{*}P < 0.05$ vs. 5 min bilateral carotid artery occlusion control, $^{**}P < 0.001$ vs. 5 min bilateral carotid artery occlusion control. Student's *t*-test.

UK). After surgery, the animals were placed in a four compartmental thermacage (Beta Medical and Scientific, UK), which maintained the environmental temperature at 28°C and rectal temperatures were measured for a 6,h period after occlusion. LY042826 and LY393615 were administered at 15 mg/kg i.p. 30 min prior to and 2 h 30 min post-occlusion in the first experiment. LY042826 and LY393615 were administered at 15 mg/kg i.p. immediately after and 3 h post-occlusion in the second experiment.

Reference compounds (−)-*trans*-1-butyl-4-(4-dimethylaminophenyl)-3-[(4-trifluoromethylphenoxy)methyl] piperidine dihydrochloride (NNC 09-0026), 4-[2-(3,4-dichlorophenoxy)ethyl]-1-pentyl piperidine hydrochloride (SB 201823-A), 2-amino-1-(2,5-dimethoxyphenyl)-5-trifluoromethyl benzimidazole (NS 649) and *N*-acenaphthyl-*N*′-4-methoxynaphth-1-yl guanidine (CNS 1237) were synthesised at the Lilly Research Centre. NNC 09-0026 was administered at 30 mg/kg i.p. 30 min prior to, 24 h, and 48 h post-occlusion, CNS 1237 (30 mg/kg, i.p.), SB 201823-A (10 mg/kg, i.p.) and NS 649 (50 mg/kg, i.p.) were administered 30 min before and 2 h 30 min after occlusion.

Five days after surgery, the animals were perfused transcardially with 30 ml of 0.9% saline followed by 100 ml of 10% buffered formalin solution. The brains were removed and placed in 10% formalin for 3 days, processed and embedded in paraffin wax. Coronal sections (5 μm) were taken 1.5, 1.7 and 1.9 mm caudal to bregma using a microtome (Leitz 1400 sledge microtome). The sections 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 × 0.05 mm) as described previously (O'Neill et al., 1998). The neuronal density is expressed as the number of viable cells per mm CA1 hippocampus. Statistical analysis of histological data was assessed using a two-tailed unpaired Students *t*-test, with P values < 0.05 being considered statistically significant.

2.2. Focal ischaemia

2.2.1. Endothelin-1 model

Rats were anaesthetised with inhalation anaesthetic and placed on a thermostatically controlled heating blanket to maintain body temperature within the range 37–38°C. The rat was then placed in a Kopf stereotaxic frame and the scalp incised so as to reveal the parietal bones. A 28-gauge steel cannula was then inserted stereotaxically at the following coordinates (from bregma: AP = +0.9 mm, L = −5.2 mm and −8.7 mm below skull, Sharkey et al., 1993). Endothelin-1 (200 pmol in 3 μl) was infused over a 3-min period. The cannula was left in situ for a further 5 min and then withdrawn (O'Neill et al., 2000). The wounds (muscles and skin) were sutured and the rat allowed to recover.

LY042826 and LY393615 were administered at 15 mg/kg i.p. immediately post-occlusion followed by an additional dose of 15 mg/kg at 3 h post-occlusion. (5*R*,10*S*)-(+)- 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-

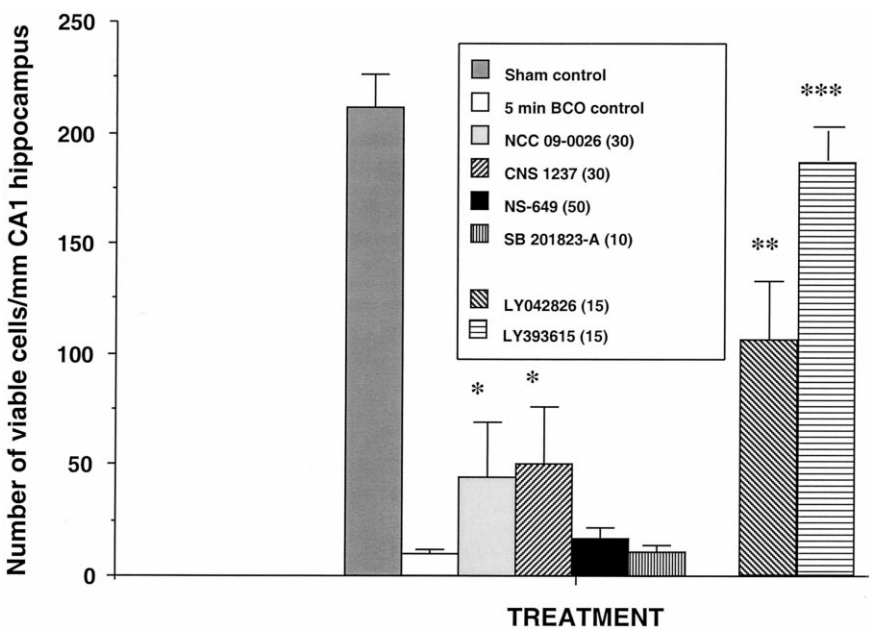

Fig. 4. Comparison of the effects of LY042826 and LY393615 with a range of small molecule Ca^{2+} channel antagonists in the gerbil model of cerebral ischaemia. All compounds were administered 30 min before occlusion. Results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampus ($n = 8$ animals per group). The 5-min bilateral carotid artery occlusion caused a severe loss in neuronal cells in the CA1 region ($P < 0.001$). NS-649 and SB 201823-A failed to protect, NCC 09-0026 and CNS1237 provided some protection, whereas LY042826 and LY393615 provided good neuroprotection against the ischaemia-induced cell death. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P <$ 0.001 vs. 5 min bilateral carotid artery occlusion control. Student's *t*-test.

cyclohepten-5,10-imine (MK-801) was administered at 2.5 mg/kg i.p. immediately after occlusion. Three days after surgery, the rats were perfused with heparinised 0.9% saline followed by 10% buffered formalin via the heart. The brains were removed and processed for histological analysis. Sections were stained with Cresyl violet, the area of ischaemic damage at eight stereotaxic levels was measured using Optimus 5.2 software and from this, an infarct volume was calculated. Statistical analysis of histological data was carried out using analysis of variance (ANOVA) followed by Students *t*-test using $P < 0.05$ as the level of significance.

3. Results

The structures of (*N*-{2-[(2-methylphenyl)(phenyl)methoxy]ethyl}-1-butanamine hydrochloride, LY042826); (*N*-{[5,5-bis(4-fluorophenyl)tetrahydro-2-furanyl]methyl}-1-butanamine hydrochloride, LY393615); ((−)-*trans*-1-butyl-4 -(4-dimethylaminophenyl)- 3 -[(4-trifluoromethylphenoxy)methyl] piperidine dihydrochloride, NNC 09-0026), (2-amino-1-(2,5-dimethoxyphenyl)-5-trifluoromethyl benzimidazole, NS-649); (*N*-acenaphthyl-*N*′-4-methoxynaphth-1-yl guanidine, CNS1237) and (4-[2-(3,4-dichlorophenoxy)ethyl]-1-pentyl piperidine hydrochloride, SB 201823-A) are illustrated in Fig 1.

For global ischaemia studies, 5-μm sections taken 1.5–1.9 mm caudal to the bregma in the anterior hippocampus were examined under a microscope with grid lines. The pyramidal cell density was counted at three different stereotaxic levels in the CA1 region of the hippocampus and the results expressed as mean ± S.E.M. neuronal density per 1 mm CA1. The results indicated that there was severe loss of neurones in the CA1 region of the hippocampus of 5-min-occluded animals. The neuronal death involved nearly all the pyramidal neurones and this neurodegeneration was not evident in any other forebrain region. In the first study, both LY042826 and LY393615 provided significant neuroprotection against the ischaemia-induced cell death in the CA1 when dosing commenced 30 min before occlusion, $P < 0.01$ and $P < 0.001$, respectively (Fig. 2).

In further studies, LY042826 ($P < 0.05$) and LY393615 ($P < 0.01$) also provided significant protection, when administered immediately after occlusion (Fig. 3).

For comparison, we evaluated the effects of other small molecule Ca^{2+} channel blockers in the gerbil model of global cerebral ischaemia. The results indicated that NNC 09-0026 protected (22%) against this ischaemia-induced cell death in the CA1 region when administered at 30 mg/kg i.p. 30 min before followed by two further doses at 24 and 48 h post-occlusion. CNS 1237 provided similar (21%) protection when administered at 30 mg/kg i.p. 30 min before and 2 h 30 min post-occlusion. In contrast, NS 649 (50 mg/kg, i.p.) and SB 201823-A (10 mg/kg, i.p.) failed to provide any neuroprotection when administered 30 min before and 2 h 30 min after occlusion. Fig. 4 summarises the effects of NCC 09-0026, CNS1237, SB 201823-A and NS-649 in the gerbil model and compares

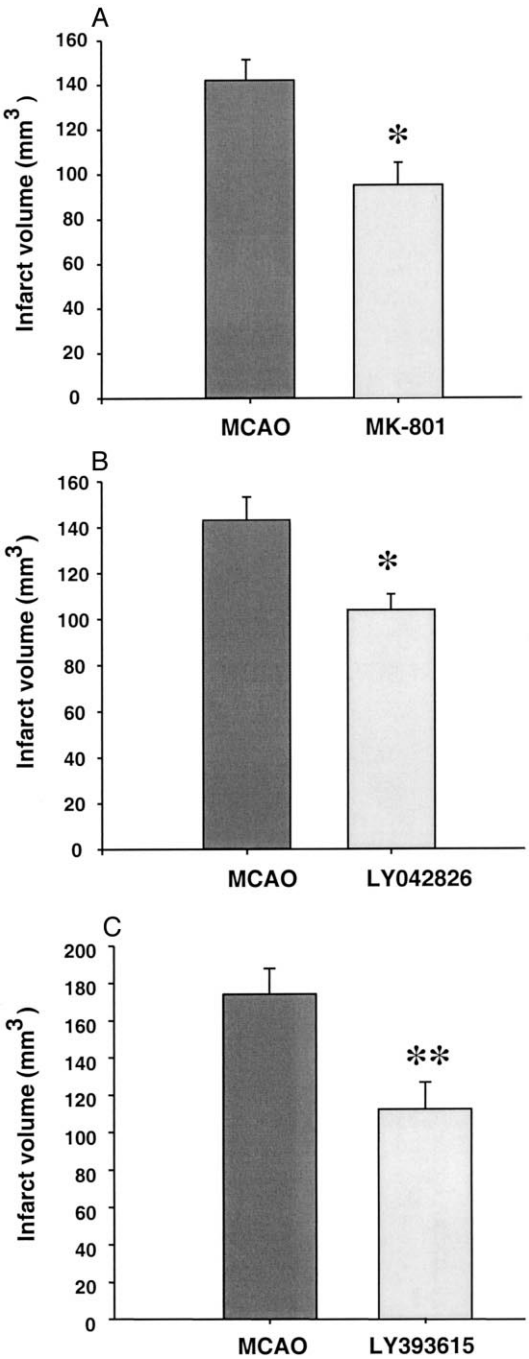

Fig. 5. Effects of MK-801, LY042826 (experiment 1) and LY393615 (experiment 2) on infarct volume (mm^3) 72 h after endothelin-induced middle cerebral artery occlusion in the rat. LY042826 and LY393615 were administered at 15 mg/kg i.p. immediately post-occlusion followed by an additional dose of 15 mg/kg at 3 h post-occlusion. MK-801 was administered at 2.5 mg/kg i.p. immediately after occlusion ($n = 10–15$ animals per group). All compounds produced a significant reduction in the infarct volume when administered immediately ($^{*}P < 0.05$, $^{**}P < 0.01$) post-occlusion. Students *t*-test.

these results to those obtained with LY042826 and LY393615.

We also evaluated the effects of LY042826 and LY393615 in the endothelin-1 model of focal cerebral ischaemia. The area of damage in mm^2 was measured at eight stereotaxic levels and the infarct volume calculated in mm^3. Both LY042826 (Fig. 5B) and LY393615 (Fig. 5C) produced a reduction in infarct volume when administration was initiated immediately and repeated 3 h after occlusion (Fig. 5). The degree of protection was similar to that observed with the NMDA receptor antagonist, MK-801 administered at 2.5 mg/kg immediately after occlusion (Fig. 5A).

4. Discussion

In the present studies, we have evaluated the effects of LY042826 and LY393615 in models of global and focal cerebral ischaemia in vivo. The results indicate that both compounds provide significant neuroprotection when administration was initiated prior to occlusion and some protection when administered immediately post-occlusion in global ischaemia. The compounds also provided greater protection than several other small molecule Ca^{2+} channel blockers in global ischaemia. Finally, LY042826 and LY393615 also had significant protective effects in focal cerebral ischaemia.

4.1. Global ischaemia

Several studies have reported that depolarization following cerebral ischaemia opens voltage-gated Ca^{2+} channels and this in turn produces massive neurotransmitter release. The large release in neurotransmitters is thought to produce excitotoxicity, free radical production, membrane degeneration and ultimately lead to cell death. In the present studies we have evaluated two new neuronal Ca^{2+} channel blockers, LY042826 and LY393615, and investigated their protective effects against ischaemic brain injury in vivo. LY042826 and LY393615 are novel neuronal Ca^{2+} channels that block Ca^{2+} flux in HEK293 cells transfected with human Ca^{2+} channel subunits. In addition to this, the IC_{50} value for Ca^{2+} currents measured by whole cell patch clamp electrophysiology in α1B and α1E transfected HEK 293 cell lines were 1.9 ± 0.1 and 5.2 ± 1.1 μM, respectively. Further studies indicate that both compounds block P-type Ca^{2+} channels in dissociated Purkinje cells and inhibit synaptic transmission in hippocampal slices (Thomas et al., 1999). The compounds also blocked sodium channels (with IC_{50} values of approximately 5 μM), but had no activity on any other receptor subtype.

Earlier studies had also reported neuroprotection with ω-conotoxin in models of transient global ischaemia in rats and gerbils (Buchan et al., 1994; Valentino et al., 1993). However, due to poor blood brain barrier penetration these peptides had to be administered centrally or intravenously at high doses, which in turn affected blood pressure. With this in mind, a small molecular Ca^{2+} channel blocker with good brain penetration would be a better candidate for treatment of ischaemic conditions. Our results indicate that two new molecules provide good protection if administered 30 min before occlusion and some protection when administration is delayed until immediately after occlusion. In previous studies, we have demonstrated that this protection is dose-dependent (O'Neill et al., 1999). The minimal effective dose was 10 mg/kg i.p., while higher doses 12.5 and 15 mg/kg i.p. provided maximal protection. In the present studies, there was a direct relationship was observed between the potency of the compounds at inhibiting Ca^{2+} channel subunits and blocking KCl-induced glutamate release in vitro and the protection afforded in vivo.

Several other small molecules, NNC 09-0026, SB 201823-A, NS 649, and CNS 1237 have also been reported to have neuroprotective effects in models of cerebral ischaemia. For example, Sheardown et al. (1993) reported that NNC 09-0026 (30 mg/kg, i.p.) protected in the gerbil when administered 30 min, 24 and 48 h post-occlusion, but lost protection when the compound (30 mg/kg, i.p.) was administered once at 30 min post-occlusion. NNC 09-0026 (30 mg/kg, i.v.) has also been reported to reduce the infarct volume in a rat model of focal ischaemia (Barone et al., 1994) when administered slowly over a 1-h period beginning 30 min post-ischaemia. Studies with CNS 1237, a use-dependent Na^+ and Ca^{2+} channel blocker, in the rat middle cerebral artery occlusion, produced a reduction in infarct volume when dosed as a bolus of 3 mg/kg i.v. followed by an infusion of 0.75 mg/kg/h for 4 h (Goldin et al., 1995). It has also been reported that SB 201823-A administered at 10 mg/kg i.p. 30 min post-occlusion attenuated ischaemia-induced hyperactivity and protected the CA1 pyramidal cells in the hippocampus following 8 min of bilateral carotid artery occlusion in the gerbil (Benham et al., 1993). Also, other studies have indicated that SB 201823-A reduces the infarct volume in rat and mouse models of focal ischaemia (Barone et al., 1995). Finally, NS-649, another neuronal Ca^{2+} channel blocker, has been reported to produce protective effects in the mouse middle cerebral artery occlusion model (Varming et al., 1996).

As a comparison, we have studied the effects of LY042826 and LY393615 and all of the above-mentioned small molecule neuronal Ca^{2+} channel antagonists in the gerbil model of global cerebral ischaemia. The doses selected were based on previously published work and their tolerability in animals. NNC 09-0026 (30 mg/kg, i.p.) provided 22% neuroprotection when administration was initiated 30 min before occlusion followed by two further doses at 24 and 48 h post-occlusion. CNS 1237 provided some neuroprotection (21%) against the ischaemia-induced cell death in the CA1 region of the hippocampus. SB 201823-A, when administered at 10 mg/kg 30 min before

and 2 h 30 min after occlusion failed to provide any neuroprotection and NS-649 at 50 mg/kg i.p. dosed 30 min before and again at 2 h 30 min after occlusion, also failed to provide any protection against the ischaemia-induced damage in the CA1 hippocampal region of the gerbil brain. We also examined the effects of these comparitor compounds on HEK293 cells transfected with human α1A, α1 or α1E Ca^{2+} channel subunits and the results indicated that concentrations of 30 μM were required to block Ca^{2+} flux. This may provide an explanation for the poor protection observed with these molecules. The present results clearly indicate that LY042826 and LY393615 are superior to the other small molecule neuronal Ca^{2+} channel blockers as neuroprotective agents in global cerebral ischaemia. The compounds exhibited good solubility and penetrated the brain well (i.e., 30 min after 10 mg/kg i.p. injection the brain levels of LY393615 were 17.5 ± 4.54 μM; O'Neill et al., unpublished results), and this probably accounts for the significant neuroprotection we observed. In the present studies, we did not measure brain temperature, but rectal temperature was measured every 30 min. We observed no changes in rectal temperature at the does that afforded neuroprotection and indeed the animals were maintained in temperature regulated cages for at least 6 h after ischaemia. Another variable with neuroprotection studies in vivo is the effect of the test compound on blood pressure. In parallel studies, we found that there was no changes in blood pressure using a dose of 15 mg/kg i.p. Therefore, the neuroprotective actions of LY042826 and LY393615 appear to be mediated by Ca^{2+} and/or sodium channel blockade.

4.2. Focal ischaemia

We also evaluated the effects of LY042826 and LY393615 in the endothelin-1 model of middle cerebral artery occlusion in the rat. The results indicated that LY042826 and LY393615 (15 mg/kg, i.p.) provided a significant reduction in infarct volume when administered immediately after and repeated 3 h post-occlusion in this model. The degree of protection was similar to that observed with MK-801 (2.5 mg/kg, i.p.), dosed immediately after occlusion, which was evaluated in a parallel study and as had been previously reported in this model (Sharkey et al., 1994). In separate studies we observed a small non-significant protective effect (using a monofilament model of focal ischaemia) with LY393615, when dosing was delayed until 2 h after occlusion (O'Neill et al., 1999). Other studies have reported that the conotoxin MVIIA, which selectively blocks N-type Ca^{2+} channels, effectively reduces infarct volume in rat models of focal cerebral ischaemia when administered during (Takizawa et al., 1995) or after the occlusion (Buchan et al., 1994; Yenari et al., 1996). Omega conotoxin MVIIA has also been evaluated in spontaneously hypertensive rats subjected to 60 min of focal cerebral ischaemia (Bowersox et al., 1997) significantly reducing cortical infarct volumes when intravenous infusion (167 μg/kg/min for 30 min) was initiated immediately after reperfusion. This compound was also reported to have neuroprotective effects in a rabbit model of focal ischaemia (Perez-Pinzon et al., 1997). More recently, ω-conotoxin MVIIA (ziconotide), has also been shown to have neuroprotective effects in a rat model of spinal ischaemia (Bruns et al., 1999). Other studies have demonstrated that i.c.v. administration of ω-agatoxin IVA, which blocks P/Q-type Ca^{2+} channels, protects against focal ischaemia in rats (Asakura et al., 1997). Other small organic molecules that block neuronal Ca^{2+} channels, such as SB 201823A have been evaluated in models of focal ischaemia and have been shown to be protective (Barone et al., 1994, 1995), the newer compound SB 206284A afforded protection when administered 4 h post-occlusion in a photochemical model of focal ischaemia (Wood et al., 1997).

In summary, the present studies demonstrate neuroprotective effects with LY042826 and LY393615 in the gerbil model of global and in the rat endothelin-1 model of focal cerebral ischaemia. In comparison with other neuronal Ca^{2+} channel blockers, in the gerbil model of ischaemia, LY042826 and LY393615 provided a superior neuroprotection which, was afforded even when dosing was delayed until after the ischaemic insult. Both LY042826 and LY393615 provided a significant reduction in infarct size when tested in the endothelin-1 focal model and compared directly with the neuroprotection afforded by MK-801 in this model. We have shown that these novel Ca^{2+} channel blockers, with good solubility and brain penetration, protect against ischaemic-induced brain injury in two models of ischaemia. Both compounds provided neuroprotection when administration was delayed until post-ischaemia and may therefore be useful anti-ischaemic agents.

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