LY393615, a novel neuronal Ca\(^{2+}\) and Na\(^+\) channel blocker with neuroprotective effects in models of in vitro and in vivo cerebral ischemia

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Abstract

In the present studies we have examined the effects of a new calcium channel blocker, LY393615 ((N-Butyl-[5,5-bis-(4-fluoro-phenyl)tetrahydrofuran-2-yl][methylamine hydrochloride, NCC1048) in a model of hypoxia–hypoglycaemia in vitro and in a gerbil model of global and in two rat models of focal cerebral ischaemia in vivo. Results indicated that LY393615 protected against hypoxia–hypoglycaemic insults in brain slices and also provided significant protection against ischaemia-induced hippocampal damage in gerbil global cerebral ischaemia when dosed at 10, 12.5 (\(P<0.05\)) or 15 mg/kg i.p. (\(P<0.01\)) 30 min before and 2 h 30 min after occlusion. The compound penetrated the brain well after a 15 mg/kg i.p. dose and had a half-life of 2.5 h. In further studies LY393615 was protective 1 h post-occlusion when administered at 15 mg/kg i.p. followed by 2 doses of 5 mg/kg i.p. 2 and 3 h later. LY393615 dosed at 15 mg/kg i.p. followed by 2 further doses of 5 mg/kg i.p. (2 and 3 h later) also produced a significant reduction in the infarct volume following Endothelin-1 (Et-1) middle cerebral artery occlusion in the rat when administration was initiated immediately (\(P<0.01\)) or 1 h (\(P<0.05\)) after occlusion. The compound was also evaluated in the intraluminal monofilament model of focal ischaemia. The animals had the middle cerebral artery occluded for 2 h, and 15 min after reperfusion LY393615 was administered at 15 mg/kg i.p. followed by 2 mg/kg/h i.v. infusion for 6 h. There was no reduction in infarct volume using this dosing protocol. In conclusion, in the present studies we have reported that a novel calcium channel blocker, LY393615, with good bioavailability protects against neuronal damage caused by hypoxia–hypoglycaemia in vitro and both global and focal cerebral ischaemia in vivo. The compound is neuroprotective when administered post-occlusion and may therefore be a useful anti-ischaemic agent.

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1. Introduction

Although cerebral ischaemia is of major clinical importance in stroke and cerebrovascular disorders, the exact mechanism of ischaemia-induced neuronal cell death remains to be elucidated. It is thought that during ischaemia, the lack of energy to the brain may depolarize neurones and result in large increases in neurotransmitters such as glutamate, aspartate, dopamine and serotonin [13,39,40]. Glutamate, through an action on N-methyl-D-aspartate (NMDA) and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, allows calcium to enter the
cell [10,22,51]. Glutamate can also act on metabotropic receptors leading to the production of diacylglycerol (DAG) and inositol triphosphate (IP3) which, lead to the release of calcium from intracellular stores [51]. Calcium also enters neurones through voltage-dependent calcium channels which open in response to cellular depolarization [39,40]. The net result of these various mechanisms by which calcium concentrations are elevated is a calcium ‘overload’ which leads to activation of proteases, nucleases, phospholipases, NO synthase and other degradative enzymes that lead to free radical production, mitochondrial degeneration and cell death [39,40]. In addition, recent studies have shown that apoptotic mechanisms contribute to cell death in vitro and damage following cerebral ischaemia in vivo [17]. Therefore, strategies aimed at inhibiting apoptosis (caspases inhibitors, etc.) may also be useful interventions in ischaemic situations (for review see [25,50]).

In line with the above mechanisms, several studies have reported that compounds acting as antagonists of excitatory amino acid receptors have beneficial effects against ischaemic insults [11,12,22,28,36]. However, several early studies also reported neuroprotection with compounds such as (S)-enamoomit, which belong to the phenylalkylamine class of calcium channel inhibitors, in animal models of cerebral ischaemia [18,23,24]. In addition, other L-type calcium antagonists such as nimodipine and nicardipine have been shown to display neuroprotective effects in some animal models of cerebral ischaemia [1,15,33]. At least 5 subtypes of high threshold (HVA) denoted L, N, P, Q and R and one type of low threshold (LVA), the T-type, voltage-dependent calcium channels have been described [47]. The availability of several synthetic conopeptides has provided an opportunity to evaluate the therapeutic potential of selective blockade of N-type calcium channels in a variety of pathological conditions including cerebral ischaemia. A single bolus intravenous administration of ω-conotoxin MVIIA (SNX-111) provided protection even when administered 24 h after the ischaemic insult [48]. Similar effects were observed in two other studies [42,55]. SNX-111 has also been found to be highly effective in reducing the necocortical infarct volume in rat models of focal ischaemia, both when administered during the occlusion [43] and after the ischaemic episode [9]. Buchan et al. [9] administered SNX-111 at 5 mg/kg i.v. either 6 or 24 h after 10 min of 4-vessel occlusion (4VO) and found protection in both cases [9]. The group also showed protection with the same dose in a rat model of focal ischemia.

More recent studies have reported neuroprotective effects with smaller non-peptide compounds that inhibit neuronal calcium channels. It has been reported that NNC 09-0026 inhibits neuronal voltage-dependent calcium channels without cardiovascular effects and provides neuroprotective effects in animal models of cerebral ischaemia [3,37]. SB 201823-A is another non-peptide calcium channel blocker that exhibits broad activity against neuronal calcium channels and at 10 mg/kg i.p. protected against global ischaemia-induced brain damage in the gerbil when administered 30 min after occlusion [4,7]. More recent studies have reported that SB 201823-A antagonises calcium currents in rat central neurones and reduces the infarct volume in rat and mouse models of focal ischaemia [4] and that another neuronal calcium antagonist, NS-649, has provided protective effects in the mouse MCAO model [49].

At Eli Lilly & Co. Ltd. we ran a high throughput screen using HEK293 cells transfected with human α1A, α1B and α1E calcium channel subunits (representing N, P/Q and R-type calcium channels). The aim of this screen was to discover small organic molecules that block neuronal calcium channels and thereby modulate neurotransmitter release in disease situations. We identified a compound that the medicinal chemistry department modified to the more active compound LY393615 (NCC1048), which inhibited N, P and Q-type calcium channels. At a concentration of 10 μM, LY393615 produced 75±16%, 86±8% and 95±3% inhibition of calcium flux in HEK 293 cells transfected with human α1A, α1B or α1E calcium channel subunits. The compound was also evaluated on Ca2+ currents in HEK 293 cells transfected with human α1B or α1E Ca2+ channel subunits and on P-type calcium channels in isolated Purkinje cells using voltage patch clamp techniques. LY393615 inhibited peak Ca2+ currents dose-dependently with IC50 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 LY393615 in an in vitro hypoxia–hypoglycemic slice model and in gerbil global ischaemia and two models of rat focal cerebral ischaemia in vivo.

2. Materials and methods

2.1. In vitro hypoxia–hypoglycemia

Male Wistar rats (150±50 g) were sacrificed by cervical dislocation. Coronal brain slices (350 μm thick) at the level of the striatum (typically bregma ±0.5 mm: Paxinos and Watson [30]) were incubated in chambers containing 500 ml oxygenated artificial cerebrospinal fluid (aCSF) at 34°C for 30 min. Slices were then subjected to ‘ischaemia’ by transfer to a chamber containing 500 ml deoxygenated, hypoglycaemic aCSF for 10 min [45] followed by a further 30 min of reincubation in oxygenated aCSF. Control slices were maintained in oxygenated aCSF throughout. In a proportion of cases, LY393615 (10 μM) was included in the aCSF throughout the pre-incubation, ischaemia and post-ischaemic periods.

Slices were then incubated for 30 min at 37°C with 2,3,5-triphenyltetrazolium chloride (TTC, a colorimetric index of mitochondrial enzyme activity), fixed in 10%
formalin and transferred to coverslips. Stained slices were initially scanned in true colour using an AGFA ‘Studioscan’ desktop scanner at 60 μm resolution. The ensuing images were converted to 8-bit greyscale (0–255) for mathematical analysis of staining [21]. The intensity of striatal and cortical TTC staining was analysed by Osiris™ (University Hospital of Geneva, Switzerland) or ScionImage™ (Scion Corporation) software and expressed as a mean percentage greyscale value over the whole region of interest (striatum or surrounding cortex).

Statistical comparisons between groups were made by One Way ANOVA with posthoc application of the Student–Newman–Keuls test.

2.2. Pharmacokinetics

To assess exposure of LY393615, gerbils were dosed with 15 mg/kg i.p. and brains harvested at 15, 30, 45 min, 1, 2, 4, 6 and 8 h later. We also evaluated exposure in rats at the same time points after 1 mg/kg i.v. In both cases brains were weighed and then homogenised with two volumes of distilled water. A 20 μM aqueous solution of LY393613 (N-Butyl-2-[bis-(4-fluorophenyl) methoxy]ethylamine hydrochloride) was prepared for use as an internal standard. 300 μl aliquots of brain homogenate and 10 μl internal standard solution were transferred to 1.5 ml eppendorf centrifuge tubes, mixed thoroughly and extracted with 10 μl trifluoroacetic acid (TFA) and 100 μl acetonitrile. Tubes were spun in a microcentrifuge, for 5 min at 13,000 r.p.m. and the supernatant transferred to HPLC microvials for assay. LY393615 standards of 50, 20, 1, 2, 4, 6 and 8 pmol in 3 μl trifluoroacetic acid (TFA) and 100 μl acetonitrile were monitored. then inserted stereotaxically at the following co-ordinates of 120 μm below skull, Sharkey et al. [35]. 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. The wounds (muscles and skin) were sutured and the rat allowed to recover. LY393615 was administered at 15 mg/kg i.p. immediately or 1 h post-occlusion followed by additional doses of 5 mg/kg at 2 and 3 h after the initial injection. 3 days after surgery, the rats were perfused with heparinised saline
follow by 10% buffered formalin via the heart. The brains were removed for histology, stained with cresyl violet and the area of ischaemic damage at 8 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 ANOVA followed by Students t-test using $P<0.05$ as the level of significance.

### 2.4.2. Monofilament model

In the monofilament model, a segment of 220 $\mu$m diameter nylon monofilament coated with nail polish at the tip (370 $\mu$m), was inserted into the internal carotid artery of each male Wistar rat (295–315 g), and advanced until it blocked the origin of the middle cerebral artery (Belayev et al. [6]; Zea Longa et al. [54]). After 2 h, the monofilament was retracted and reperfusion occurred. LY393615 was administered at 15 mg/kg i.p. 15 min after reperfusion followed by 2 mg/kg/h i.v. infusion for 6 h. Twenty-four hours later the brains were removed, frozen, sliced into 30 $\mu$m sections and stained with cresyl violet. The infarct area of each slice was measured with imaging system software ImagePro Plus, and infarct volumes were calculated. Statistical analysis of histological data was carried out using ANOVA followed by Students t-test using $P<0.05$ as the level of significance.

### 3. Results

The structure of (N-Butyl-[5,5-bis-(4-fluorophenyl)tetra-hydrofuran-2-yl]methylamine hydrochloride, LY393615) is illustrated in Fig. 1.

Figs. 2 and 3 show the effects of LY 393615 against ischaemic insult in vitro. Control slices mostly showed uniform staining throughout the cortex and striatum following TTC. Fig. 2 consists of representative pseudo-coloured corticostriatal sections showing the effect of ischaemia and LY393615. Ischaemia (10 min) caused a significant ($P<0.05$) reduction in mean TTC staining intensity in both striatum and cerebral cortex (Fig. 3). LY393615 had no significant effect on TTC staining intensity in control slices (if anything it appeared to protect slice) but prevented the reduction in staining induced by ‘ischaemia’ in both striatum ($P<0.01$) and cortex ($P<0.001$).

For global ischaemia studies, 5 $\mu$m 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. LY393615 provided dose-dependent neuroprotection against the ischaemia-induced cell death in the CA1. Thus, LY393615 provided very good protection when administered at 15 mg/kg i.p. 30 min before and 2 h 30 min after occlusion (Figs. 4 and 5). We also evaluated the effects of lower doses of LY393615 at 10 mg/kg or 12.5 mg/kg i.p. 30 min before and 2 h 30 min post-occlusion. Results indicated that 10 mg/kg provided a small, but significant neuroprotection (Fig. 4). The intermediate dose (12.5 mg/kg i.p.) also provided a significant neuroprotective effect (Fig. 4).

We then went on to study the pharmacokinetics (at 15, 30, 45 min, 1, 2, 4, 6 and 8 h) of LY393615 in the gerbil brain after 15 mg/kg i.p. The results indicated that the compound crossed the blood–brain barrier quickly and was detectable in the brain as early as 15 min after injection (Fig. 6). Maximal levels were observed at 1–2 h after injection and the half-life was approximately 2.5 h.

Based on the pharmacokinetic studies we postulated that a loading dose (15 mg/kg i.p.) followed by two further ‘top-up’ doses (5 mg/kg i.p.) would give relatively consistent high brain levels for several hours after injection. Therefore, in the next study we evaluated the effects of 15 mg/kg i.p. 30 min after occlusion followed by 2 further doses of 5 mg/kg i.p. at 2 h 30 min and 3 h 30 min post-occlusion. Histological results indicated that this dosing protocol also provided good ($P<0.01$) neuroprotection (Fig. 7). We then determined if the time window could be extended using the above protocol by administering the LY393615 at 15 mg/kg 1 h post-occlusion followed by 5 mg/kg at 2 and 3 h post-occlusion. Results indicated that this dosing protocol also provided significant ($P<0.05$) neuroprotection (Fig. 8).

Two models of focal cerebral ischaemia were carried out; the endothelin-1 model and the monofilament model. In the endothelin-1 model LY393615 was administered at 15 mg/kg i.p. either immediately or 1 h after endothelin-1 injection onto the middle cerebral artery in the rat and followed by two further doses at 3 and 4 h after the initial injection. The area of damage in mm$^2$ was measured at 8 stereotaxic levels and the infarct volume calculated in mm$^3$. LY393615 produced a reduction ($P<0.01$) in infarct volume when administration was initiated.
Fig. 2. Representative greyscale scans showing TTC staining of the striatum and cortex in control and ischaemia-treated slices, alone and in the presence of LY393615 (10 μM). Pinker tissue is an indicator of higher mitochondrial enzyme activity.
immediately after occlusion (Fig. 9). The compound also provided some protection ($P<0.05$) when administration was initiated 1 h post-occlusion (Fig. 9).

Pharmacokinetic studies in rats indicated that LY393615 penetrated the brain rapidly after a 1 mg/kg i.v. dose (Fig. 10). The half-life was approximately 2 h and therefore an infusion would give most consistent brain levels for further focal ischaemia studies.

LY393615 was also examined in the monolaminate model of cerebral ischaemia. The animals had the middle cerebral artery occluded for 2 h. Fifteen minutes after reperfusion LY393615 was administered at 15 mg/kg i.p. followed by 2 mg/kg/h i.v. infusion for 6 h. The results indicated that there was no significant reduction in infarct volume (Fig. 11).

### 4. Discussion

The in vitro profile of LY33165 is summarised in Table 1. At a concentration of 10 $\mu$M, LY393615 produced $75\pm16\%$, $86\pm8\%$ and $95\pm3\%$ inhibition of calcium flux in HEK 293 cells transfected with $\alpha$1A, $\alpha$1B or $\alpha$1E calcium channel subunits. In vitro electrophysiological studies using patch clamp techniques indicated that LY393165 inhibited peak Ca$^{2+}$ currents dose-dependently with IC$_{50}$ values of 1.9$\pm$0.1 $\mu$M in $\alpha$1B cells, 5.2$\pm$1.1 $\mu$M in $\alpha$1E cells and 4.0$\pm$1.0 $\mu$M in Purkinje cells. In the present studies we have evaluated the effects of LY393615 in models of in vitro and in vivo cerebral ischaemia. The results indicate that LY393615 was protective in vitro and provided excellent protection when administration was initiated prior to occlusion and good protection when administered 30 min post-occlusion in global ischaemia. The compound also had significant protective effects when delayed until 1 h post-occlusion in both global and focal cerebral ischaemia.

#### 4.1. In vitro ischaemia

Several studies have reported that depolarization following cerebral ischaemia opens voltage-gated calcium 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. We have previously shown that $\omega$-conotoxin GVIA and $\omega$-agatoxin IVA delay neurotransmitter release in striatal slices, while in contrast nimodipine and nicardipine do not [46]. Therefore, N-and P/Q-, but not L-type calcium channels seem to control this ischaemia-induced neurotransmitter release. In the present studies we have used a selective neuronal calcium channel blocker, LY393615, and investigated if this compound provides protection against hypoxia-hypoglycaemia damage in vitro. LY393615 has been shown to block calcium currents in HEK293 cells transfected with human $\alpha$1A, $\alpha$1B and $\alpha$1E Ca$^{2+}$ channel subunits, P-type calcium channels in dissociated Purkinje cells and inhibit synaptic transmission in hippocampal slices [44]. The results of the present studies indicate that LY393615 provided significant protection against hypoxic–hypoglycaemic insult in striatal slices using 2,3,5-triphenyltetrazolium chloride staining [21]. Other recent studies have reported that $\omega$-conotoxin (selective N-type blocker) protects against hypoxia induced neurodegeneration in organotypic hippocampal-slice cultures [32]. While further studies have used combinations of compounds in an attempt to identify the calcium channel subtypes contributing to neuronal injury in rat hippocampal slices subjected to oxygen and glucose deprivation [41]. These authors found that nimodipine (L-type) and daurisoline (P-type) provided less than 20% protection, $\omega$-conotoxin MVIIA (N-type) provided 40% protection and 200 nM $\omega$-agatoxin
Effects of LY391615 against Ischaemia-Induced Hippocampal Damage in the Gerbil model of Global Cerebral ischaemia

Fig. 5. The effect of LY393615 administered at 15 mg/kg i.p. 30 min before and 2 h 30 min after 5 min bilateral carotid artery occlusion (BCAO). Representative sections stained with haematoxylin and eosin of whole hippocampus from (a) sham operated, (b) 5 min BCAO control and (c) 5 min BCAO and LY393615 and animals and high power magnification from the same animals (d, e and f).
IVA (P+Q-type) provided 75% protection. However, ω-conotoxin MVIIIC (P+Q+N-type) provided complete protection, suggesting that all three neuronal calcium channels contribute to ischaemic brain injury in the hippocampus.

4.2. Global ischaemia

Earlier studies had also reported neuroprotection with ω-conotoxin GVIA (SNX-111) in models of transient global ischaemia in rats and gerbils [9,48]. However, these peptides had poor blood brain barrier penetration and have to be administered centrally or intravenously at high doses, which effected blood pressure. Therefore, a small molecular calcium channel blocker with good brain penetration would be a better candidate for treatment of ischaemic conditions. We have demonstrated that LY393615 provided good neuroprotection when dosed at 15 mg/kg i.p., 30 min before occlusion in the gerbil model. This protection was dose-dependent, with the 15 mg/kg dose providing much greater protection than the lower 10 and 12.5 mg/kg doses. Pharmacokinetic studies indicated that the compound penetrated into the brain and that the half-life was approximately 2.5 h. Based on these studies, we calculated that 15 mg/kg i.p. followed by two further doses of 5 mg/kg 2 and 3 h later would give consistent brain levels that were several fold higher than the IC50 for LY393615 on
neuronal calcium channel subunits in vitro. Using this protocol we went on to demonstrate that LY393615 provided significant protection in this model when the first injection was delayed until 1 h post-occlusion.

Several other small molecules; NNC 09-0026 ((−)-trans-1-butyl-4-(4-dimethylaminophenyl)-3-[(4-trifluoro-methylphenoxy)methyl]piperidine dihydro chloride); SB 201823-A (4-[2-(3,4-dichlorophenoxy)ethyl]-1-pentyl piperidine hydrochloride); NS 649 (2-amino-1-(2,5-dimethoxyphenyl)-5-trifluoromethyl benzimidazole); CNS 1237 (N-acenaphthyl-N°-4-methoxynaphth-1-yl guanidine) have also been reported to have neuroprotective effects in models of cerebral ischaemia. For example, Sheardown and co-workers [38] have reported that NNC 09-0026 (30 mg/kg i.p.) protected in the gerbil when administered 30 min, 24 and 48 h post-occlusion [37], but no neuroprotec-

![Graph](image-url)

**Fig. 10.** Brain levels of LY393615 (1 mg/kg i.v.) at 15, 30, 45 min, 1, 2, 4, 6 and 8 h after dosing in rats. Results indicate that LY393615 penetrates the brain rapidly has a half-life of approximately 2 h.

### Table 1

The in vitro profile of LY393615a

| (A) Inhibition of Ca²⁺ flux at 10 mm using Fluoroscan: |
| HEK 293 cells transfected with human α1A | 75±16 |
| HEK 293 cells transfected with human α1B | 86±8 |
| HEK 293 cells transfected with human α1E | 95±3 |

| (B) Inhibition of Glutamate release at 10 μm: |
| KC1-induced glutamate release | 76±6 |
| Electrical-induced glutamate release | 63 (n = 2) |
| Veratradine-induced glutamate release | 81 (n = 2) |

| (C) Patch Clamp IC₅₀ values (μm) for inhibition of: |
| HEK 293 cells transfected with human α1B | 1.9±0.1 |
| HEK 293 cells transfected with human α1E | 5.2±1.1 |

P-type calcium channels in isolated Purkinje cells 4.0±1.0

| Effects of LY393615 ON (A) calcium flux in HEK 293 cells transfected with human α1A, α1B and α1E calcium channel subunits (B) inhibition of DC1 Electrical and Veratradine-induced glutamate release in synaptosomes and (C) IC₅₀ values for inhibition of calcium channels in transferred cell lines and isolated Purkinje cells using whole cell patch clamp electrophysiological techniques.

![Graph](image-url)

**Fig. 11.** Effects of LY393615 on infarct volume (mm³) after 2 h of middle cerebral artery occlusion in the rat using the monofilament method. LY393615 was administered at a loading dose of 15 mg/kg i.p. 15 min after reperfusion followed by an i.v. infusion of 2 mg/kg/h for 6 h and infarct volume measured at 1 day post-surgery (n = 12–16 animals per group). LY393615 failed to reduce the infarct volume under this dosing protocol. Students t-test.
channel blockers as neuroprotective agents in global cerebral ischaemia. The compound exhibited good solubility and penetrated the brain well and this probably accounts for the good neuroprotection we observed.

In the past 5 years we have evaluated several pharmacological interventions in cerebral ischaemia. As mentioned above, the level of protection observed with LY393615 in this model is much greater than that we have previously observed, using the same protocol, with other calcium channel blockers (NCC 09-0026, CNS1237 [26]). Interestingly, the protection with LY393615 is also much greater than that provided by competitive NMDA antagonists (CGS19755, CPP [28]; polyamine site antagonists of the NMDA receptor [5] and glycine site antagonists (ACEA1021, GV150526A [16]), non-competitive AMPA antagonists (GYKI52466, LY300164 [20]), nitric oxide inhibitors (l-NAME and 7-nitroindazole [27]) and similar to what we have seen with the competitive AMPA antagonists (LY302679; LY293558 [28]), the iGlur5 antagonist (LY377770 [29]) and the mGlur2/3 agonist (LY379268 [8]). Therefore, LY393615 has comparable neuroprotection to some of the best compounds we have evaluated to date in the gerbil model of cerebral ischaemia.

4.3. Focal ischaemia

In focal ischaemia studies we evaluated the neuroprotective potential of LY393615 in the endothelin-1 and monofilament models of middle cerebral artery occlusion (MCAO) in rats. The results indicated that LY393615 provided a significant reduction in infarct volume when administered immediately or 1 h post-occlusion in the endothelin-1 model of focal cerebral ischaemia. The degree of protection was similar to that previously reported with MK-801 in this model [29,34]. LY393615 failed to protect when administered at 15 mg/kg i.p. 15 min after 2 h of MCAO and followed by a 6 h infusion of 2 mg/kg i.v. for 6 h in the monofilament model. However, the compound was administered 2 h 15 min after ischaemia and further pharmacokinetic studies indicated that although LY393615 penetrated the brain following i.v. administration, it has a short half-life (2.04 h) and, therefore, a higher infusion dose (3.5 mg/kg/h) may have been more optimal to achieve maximal chances of protection. Other studies have reported that the conotoxin SNX-111, which blocks N-type calcium channels is effective at reducing infarct volume in rat models of focal cerebral ischaemia when administered during [43] or after the occlusion (Buchan et al. [9]; Yenari et al. [53]). The compound was also reported to have neuroprotective effects in a rabbit model of focal ischaemia [31]. Other recent studies have demonstrated that i.c.v. administration of ω-agatoxin IVA, which blocks P/Q-type calcium channels, protects against focal ischaemia in rats [2]. However, other investigators have demonstrated that another P-type calcium antagonist, daurisoline (a non-peptide) failed to protect in a rat model of focal ischaemia [19]. More recent work has indicated that small organic molecules such as SB 201823A are protective in models of focal ischaemia [3,4]. The newer compound SB 206284A has been reported to protect when administered 4 h post-occlusion in a photochemical model of focal ischaemia [52]. However, many of the studies to date have used short occlusion times i.e. 90 min whereas in our studies we have used the standard 2 h occlusion, which is a more severe model.

4.4. Mechanism of action of LY393615

As mentioned above, LY393615 blocks several types of neuronal calcium channels and also inhibits sodium channels. Therefore, the exact mechanism of neuroprotection is not clear. In theory all of the activities (i.e. inhibition of N-type, P/Q-type of calcium channels or sodium channels) could be neuroprotective. On reviewing the literature it is clear that selective N-type calcium channel blockers and sodium channel blockers have provided consistent protection in a variety of models. However, results are more variable with P/Q-type calcium blockers. Therefore, it is most likely that the N-type calcium channel blockade and the sodium channel effects account for the observed neuroprotection. It is interesting to note that there is an increasing tendency to screen for selective molecules using transfected cell lines, but there are multiple receptors contributing to ischaemia-induced brain damage. The pharmacological profile of LY393615 may make it more suitable as a neuroprotectant than a compound that only blocked one receptor subtype. In the present studies we did not measure brain temperature and it is well established that hypothermia is neuroprotective. We did monitor rectal temperatures at 30 min intervals and this is thought to be a good reflection of brain temperature. The compound did not alter rectal temperature at the neuroprotective doses. In a similar way the protection observed could be due to effect 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 LY393615 appear to be mediated by calcium and/or sodium channel blockade.

In summary, in the present studies we have reported neuroprotective effects with LY393615 a model of in vitro ischaemia in striatal slices and in models of global and focal cerebral ischaemia in vivo. The compound had good solubility and pharmacokinetic studies demonstrated that the compound had good brain penetration with a half-life of 2–2.5 h. LY393615 was active post-occlusion and may therefore be useful as an anti-ischaemic agent.

References


