British Journal of Pharmacology (1995) 116, 3093-3098

Neuroprotective efficacy of lifarizine (RS-87476) in a simplified rat survival model of 2 vessel occlusion

[1]Douglas E. McBean, Victoria Winters, Alasdair D. Wilson, Craig B. Oswald, Brian J. Alps & J. Michael Armstrong

Department of Pharmacology, Syntex Research Centre, Research Avenue South, Heriot Watt University Research Park, Riccarton, Edinburgh, EH14 4AP

1 A new, modified rat two vessel occlusion model (with hypotension) was established and the neuroprotective efficacy of the novel agent lifarizine (RS-87476) was examined.

2 The two vessel occlusion model used in the study was a modification of the model described in the literature, whereby we have obviated the need to use a muscle relaxant and intubate the trachea to provide ventilatory support by providing a tight fitting face mask attached to the ventilator. Furthermore, the need to combine exsanguination and additional pharmacological means of inducing the mandatory hypotension (50 mmHg), required to decrease brain blood perfusion pressure, has been removed by simply manipulating the concentration of the already present halothane anaesthetic.

3 The appropriate level of hypotension having been reached, microvascular clips were applied to bilaterally occlude the common carotid arteries for 12 min. This resulted in a loss of the cortical EEG activity. Local cerebral blood flow was measured 6 min into the occlusion period, using the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique, in a separate group of rats ($n=5$). This illustrated the lack of any blood flow, in the areas under study, during the period when there was an isoelectric cortical EEG pattern.

4 The high grade global ischaemic lesion which occurred gave quantifiable neuronal damage in several vulnerable regions of the brain, namely, the hippocampal CA_1 sub-field, cortex, thalamus, striatum, and cerebellar brain stem (Purkinje cells).

5 Following the global ischaemic insult the rats were allowed to recover for 72 h before assessment of the damage, during which time one group of rats ($n=11$) received 100 μg kg^{-1} lifarizine i.a. 5 min post-occlusion, 500 μg kg^{-1} lifarizine i.p. 15 min post-occlusion, and 500 μg kg^{-1} lifarizine i.p. twice daily for 72 h. A second group of rats ($n=12$) was treated with appropriate volumes of vehicle (0.4 ml kg^{-1} i.a. and 2 ml kg^{-1} i.p.) at identical time points.

6 Histopathological damage was assessed, from cresyl violet and haematoxyline/eosin stained sections, using a scoring system of 0-6 (no damage - complete neuronal death). The dosing regimen of lifarizine gave reduced damage in the hippocampal CA_1 sub-field (4.1 ± 0.3 to 2.8 ± 0.6) and striatum (1.7 ± 0.3 to 1.2 ± 0.3) and significant neuroprotection in the anterior cortex (2.0 ± 0.2 to 1.2 ± 0.2; $P<0.05$), thalamus (1.5 ± 0.2 to 0.8 ± 0.2; $P<0.01$), posterior cortex (1.5 ± 0.2 to 1.0 ± 0.2; $P<0.05$) and cerebellar brain stem (0.9 ± 0.2 to 0.4 ± 0.1; $P<0.01$). The overall mean brain score was significantly reduced (from 1.5 ± 0.1 to 0.9 ± 0.2).

7 These data show that the newly modified 2 vessel occlusion model produced a quantifiable level of ischaemic damage and that the novel agent lifarizine is neuroprotective in the model.

Keywords: Global ischaemia; rat 2 vessel occlusion; anaesthetic induced hypotension; neuropathological score; lifarizine

Introduction

The rat two vessel occlusion (2VO) survival model of forebrain ischaemia was first proposed over 20 years ago (Eklof & Siesjo, 1972), and has received increasing attention in recent years as an alternative to the rat four vessel occlusion (4VO) model (Pulsinelli & Brierley, 1979) with which it has been compared. The aim of the 2VO model is to produce a bilateral cerebral ischaemic insult where both the onset and reversal of ischaemia are rapid (Smith *et al.*, 1984). The insult gives rise to ischaemic cell change (ICC) in a variety of selectively vulnerable areas such as the CA_1 sub-field of the hippocampus, striatum and neocortex, producing a general histopathology similar to that of the 4VO model (Pulsinelli & Brierley, 1979).

The 2VO model, as described by Smith *et al.* (1984), is carried out under general anaesthesia and requires the administration of a muscle relaxant such as suxamethonium to facilitate endotracheal intubation and mechanical pulmonary ventilation. Bilateral occlusion of the common carotid arteries alone is insufficient to reduce cerebral blood flow below the ischaemic threshold in normotensive rats (Eklof & Siesjo, 1973) or to upset the energy state of the brain tissue (Eklof & Siesjo, 1972) to the extent necessary to cause quantifiable cell death. The production of a damaging ischaemic insult is essentially dependent upon causing inadequate brain tissue blood perfusion by inducing systemic hypotension to help reduce brain blood flow when occluding the carotid arteries. This hypotensive effect is produced either by controlled exsanguination (with subsequent re-infusion), by the adjunctive administration of peripheral vasodilators, or by a combination of both approaches. The most common approach now is to produce the hypotension by exsanguination.

The purpose of this paper is to describe modifications made to the 2VO models previously studied (Eklof & Siesjo, 1972; 1973; Smith *et al.*, 1984). The need to co-administer a muscle

[1]Author for correspondence at present address: Department of Pharmacology, Quintiles Scotland Ltd., Research Avenue South, Heriot Watt University Research Park, Riccarton, Edinburgh, EH14 4AP.

relaxant and intubate the trachea is removed, and it is not necessary to exsanguinate additionally to the anaesthetic halothane in order to produce the mandatory lowering of blood pressure. Furthermore, the appropriateness and usefulness of the modified model was tested by studying the beneficial effect of lifarizine, a novel Na^+/Ca^{2+} channel inhibitor and neuroprotective agent in animals (Alps *et al.*, 1990). Part of this work has already been presented in abstract form to the British Pharmacological Society (McBean *et al.*, 1995a).

Methods

Animal preparation and experimental protocol

Experiments were carried out using male Sprague-Dawley (CDSD) rats, weighing 265–350 g. The rats were fasted overnight before surgery, but were allowed free access to water. Anaesthesia was induced with 5% halothane in oxygen (O_2), and maintained at a surgical level of 1.5 to 2% halothane in O_2. Surgery was carried out under aseptic conditions.

One pair of electrodes was inserted subcutaneously on either side of the skull to record the electroencephalogram (EEG) and a second pair were located laterally just above the tail to act as earth electrodes. The rat was then placed on its back and a small incision made in the tail to expose the tail artery. The tail artery was catheterized (cannula i.d. 0.50 mm, o.d. 0.63 mm) to allow continuous recording of the arterial blood pressure and removal of blood samples for blood gas and plasma glucose analysis. It was at this point that the pre-occlusion arterial blood samples were collected for blood gas and plasma analysis. A ventral midline incision was then made in the neck and the muscle retracted on either side of the trachea to expose both the right and left common carotid arteries, around which loose threads were placed. A tight-fitting face mask attached to a small animal ventilator (tidal volume 12 ml min^{-1}) was applied to the rat to give respiratory support during the subsequent period of arterial occlusion.

The halothane concentration in the O_2 was increased to 5% with a resulting decrease in mean arterial blood pressure (MABP). When the MABP had dropped below 50 mmHg, the carotid threads were lifted gently and small microvascular clips applied to both common carotid arteries. These clips were kept in place for 12 min, with MABP and EEG being monitored continuously. The time taken for the EEG activity to flatten (i.e. become isoelectric) was noted. The MABP was maintained at 50 mmHg or just below throughout the period of arterial occlusion. Measurements of local cerebral blood flow were made 6 min into the occlusion period in a second group of rats ($n=5$), using the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique (Sakurada *et al.*, 1978).

After exactly 12 min the clips were removed to permit reperfusion. At 3 min post-occlusion the halothane concentration was reduced to 0% to facilitate rapid recovery of blood pressure. At 5 min post-occlusion each rat received an intra-arterial (i.a.) dose of either 100 $\mu g\ kg^{-1}$ lifarizine ($n=11$) or the appropriate volume drug vehicle ($n=12$). Around 6 to 7 min post-occlusion the rats showed signs of respiring spontaneously and were removed from the ventilator. From 9 to 11 min post-occlusion EEG activity started to return, and the exact time at which the EEG was no longer isoelectric was noted for each animal. This allowed the duration of the isoelectric period to be calculated. It is important to note that up to the point at which EEG activity began to reappear the rat was not responsive to pain. Following return of EEG activity the halothane concentration was increased to a surgical level at about 1.5% and the neck wound repaired. At 15 min post-occlusion each rat received an intraperitoneal (i.p.) dose of either 500 $\mu g\ kg^{-1}$ lifarizine or the appropriate volume of drug vehicle. Following this second dose, arterial blood samples were again collected for post-occlusion blood gas and plasma glucose analysis. The arterial cannula in the tail was removed, the vessel fully ligated, and the wound sutured. Following surgery, each animal was housed separately under optimum recovery conditions, using surgical lamps (ambient temperature 30°C) and given free access to food and water. Each rat was thereafter dosed twice daily during the 72 h recovery period with i.p. injections of either 500 $\mu g\ kg^{-1}$ lifarizine or appropriate volumes of vehicle for controls.

Tissue processing and analysis

At the end of the 72 h recovery period, each rat was anaesthetized with pentobarbitone sodium (120 mg kg^{-1} i.p.) and thoracotomized. The cerebral circulation was flushed with heparinized saline via an intra-cardiac injection (left ventricle), and perfuse-fixed with 10% formalin. The whole heads were removed and stored overnight in 10% formalin at 4°C. The brains were removed and stored in fresh fixative for 4–5 days before trimming for histological examination. Coronal sections were taken at three levels: (1) the divergence of the optic chiasma; (2) the midpoint of the infundibulum; (3) the cerebellar brain stem. The portions were embedded in separate blocks of paraffin wax and 7 μm sections cut. Alternate sections were stained with haematoxylin/eosin (H&E) and cresyl fast violet for histological assessment of damage as described by Brown & Brierley (1968).

Data analysis

The brain regions examined involved the CA_1 sub-field of the hippocampus, anterior and posterior neocortices, thalamus, striatum, and cerebellar brain stem. The extent of the percentage ICC was assessed using a 0–6 neuropathological scoring system where: 0 = no damage and 1 = 0–10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100%; 6 = complete neuronal death, status spongiosus and vacuolation. The more commonly used 0–4 scale where 4 = 50–100% damage has been modified for this study to enable us to have greater differentiation at the upper end of the damage scale. The overall brain score was assessed for each individual animal by averaging the score for each brain area. A mean brain score for each treatment group was therefore calculated. Scores for the vehicle-treated group were compared with those for the lifarizine treated group by means of a non-parametric analysis using Mann-Whitney.

MABP, blood gas, core body temperature, plasma glucose and EEG data for the two groups were also compared using either an unpaired or a paired 2-tailed Student's *t* test. For the blood gas, core body temperature and plasma glucose data the following comparisons were made: (i) pre-occlusion against post-occlusion values for drug vehicle - treatment (paired); (ii) pre-occlusion against post-occlusion values for lifarizine treatment (paired); (iii) pre-occlusion values for the vehicle treatment against pre-occlusion values for lifarizine treatment (unpaired); (iv) post-occlusion values for drug treatment against post-occlusion values for lifarizine treatment (unpaired).

Drugs and solutions used

One ml of a stock solution of lifarizine (4 mg ml^{-1} in solution of 52.17 mg sorbitol and 1.03 mg tartaric acid made up to 1 ml with water) was added to 15 ml of sterile saline (0.9% w/v NaCl) to give a 250 $\mu g\ ml^{-1}$ dosing solution. Doses of 100 $\mu g\ kg^{-1}$ (i.a.) and 500 $\mu g\ kg^{-1}$ (i.p.) were administered to lifarizine-treated animals. The drug vehicle contained the same quantities of sorbitol and tartaric acid made up to 1 ml with water and this was diluted with saline in the same manner as the lifarizine stock solution, and identical volumes (i.a. and i.p.) were injected in the control animals.

Results

EEG data

No significant differences were observed in either the onset time or duration of isoelectricity when comparing the vehicle-treated with the lifarizine-treated groups (Table 1).

Blood pressure

No significant differences in the MABP between the vehicle-treated and lifarizine-treated groups were observed at any point during the 2VO experimental period (Figure 1).

Physiological variables

No significant differences were observed in any of the physiological variables (arterial blood gas, core body temperature and plasma glucose levels) measured either between the two groups or within groups pre- to post-occlusion (Table 2).

Table 1 EEG data monitored during 2 vessel occlusion

	Isoelectric period (s)	
Treatment	*Onset*	*Duration*
Vehicle	52±2	1259±30
Lifarizine	50±4	1218±19

All data are expressed as mean±s.e.mean. Lifarizine treated rats ($n=11$) received 100 $\mu g\,kg^{-1}$ i.a. 5 min post-occlusion, 500 $\mu g\,kg^{-1}$ i.p. 15 min post-occlusion, and 500 $\mu g\,kg^{-1}$ i.p. twice daily for 72 h. Vehicle-treated rats ($n=12$) received appropriate volumes of vehicle at identical time points. No significant differences were observed in either the onset or duration of isoelectricity between vehicle- and lifarizine-treated groups (unpaired, 2-tailed Student's t test).

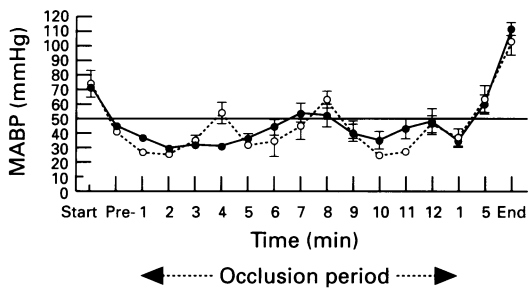

Figure 1 Mean arterial blood pressure (MABP) changes during 2 vessel occlusion experiments for both vehicle (●) ($n=12$) and lifarizine (○) ($n=11$) treated rats. The MABP (mean±s.e.mean) of vehicle and lifarizine treated rats was compared at each time point by Student's t test. At no time point was there any significant difference between the MABP of the two groups. The horizontal line (—) indicates the MABP limit of 50 mmHg, below which we were striving to keep both sets of rats during the occlusion period.

Local cerebral blood flow

No blood flow was evident in any of the structures assessed for neuropathological damage in the vehicle- or lifarizine-treated groups (hippocampal CA_1 sub-field, neocortex, thalamus, striatum, and cerebellar brain stem) when measurements were made 6 min into the occlusion period. The autoradiogram shown in Figure 2 was taken at the level of the CA_1, and illustrates the absence of blood flow in the cortex, hippocampus and lateral thalamus during the period where an isoelectric cortical EEG pattern is present in the vehicle- and lifarizine-treated rats.

Neuropathological score

The grading data are shown in Figure 3 as a scatterplot for each brain region under study. Significant reductions in the ischaemically-mediated damage were observed in the anterior neocortex (2.0±0.2 to 1.2±0.2; $P<0.05$), thalamus (1.5±0.2 to 0.8±0.2; $P<0.01$), posterior neocortex (1.5±0.2 to 1.0±0.2; $P<0.05$) and cerebellar brain stem (0.9±0.2 to 0.4±0.1; $P<0.01$). Although the scores for the hippocampal CA_1 sub-field (4.1±0.3 to 2.8±0.6) and striatum (1.7±0.3 to 1.2±0.3) were reduced in the lifarizine-treated group, they were not statistically significantly different from those determined for the control group. Overall, the mean brain score was significantly reduced following lifarizine treatment from 1.5±0.1 to 0.9±0.2 ($P<0.01$).

Discussion

There have been progressive efforts to reduce the methodological complexity and resultant surgical stress on animals expected to survive the production of a high grade global central lesion which, acutely, can be seriously exacerbated by non-controlled, non-ischaemic interventions such as excessive immediate post operative handling and stimulation. The potential disadvantage cited against the use of general anaesthesia, even with the original version of the rat 2VO model, is not really valid in our opinion. The real measurable longer term outcome of the global cerebral lesion following its resolution is impact on learning and spatial navigation, rather than the neurological deficits in locomotion more often evident with models of focal cerebral ischaemia.

Global or forebrain cerebral ischaemia can also be induced in rats by a method of 4VO as described by Pulsinelli & Brierley (1979). The use of this model has raised several issues over the years, most of which have been previously addressed (Ginsberg & Busto, 1989). The first step to readdress some of these problems involved a modification of the 4VO procedure as described by Alps & Hass (1987) for studies involving the calcium antagonist nicardipine. Here, the second stage of the procedure was carried out under gaseous anaesthetic control,

Table 2 Physiological variables monitored during 2 vessel occlusion

	Vehicle		*Lifarizine*	
Parameter	*Pre-occlusion*	*Post-occlusion*	*Pre-occlusion*	*Post-occlusion*
pH	7.30±0.01	7.30±0.01	7.30±0.01	7.30±0.01
P_{CO_2} (mmHg)	53±4	46±2	52±2	46±2
P_{O_2} (mmHg)	392±37	400±37	401±41	385±34
HCO^{3-} (mM)	23.7±0.4	22.0±0.4	23.9±0.5	21.7±0.5
Base excess (mM)	0.4±0.5	2.2±0.4	0.6±0.6	2.5±0.6
Temperature (°C)	37.3±0.1	37.7±0.2	37.3±0.1	38.0±0.1
Plasma glucose ($g\,l^{-1}$)	1.2±0.1	0.9±0.1	1.2±0.1	1.2±0.1

All data are expressed as mean±s.e.mean. Lifarizine-treated rats ($n=11$) received 100 $\mu g\,kg^{-1}$ i.a. 5 min post-occlusion, 500 $\mu g\,kg^{-1}$ i.p. 15 min post-occlusion, and 500 $\mu g\,kg^{-1}$ i.p. twice daily for 72 h. Vehicle-treated rats ($n=12$) received appropriate volumes of vehicle at identical time points. No significant differences were observed between pre- and post-occlusion for any parameter in either the vehicle- or lifarizine-treated groups (paired or unpaired, 2-tailed Student's t test).

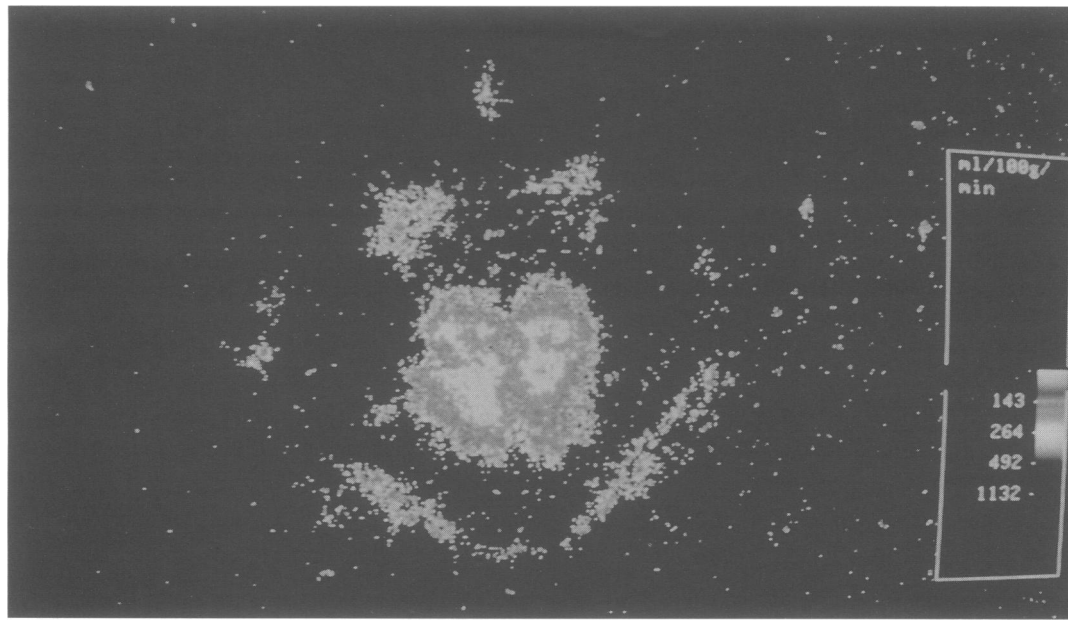

Figure 2 This [^{14}C]-iodoantipyrine autoradiogram was taken at the level of the hippocampal CA_1 sub-field of the hippocampus 6 min into the occlusion period. It can be seen that there is no blood flow in any of the areas examined for histopathological damage after 2 vessel occlusion, namely, the anterior cortex, CA_1, and thalamus.

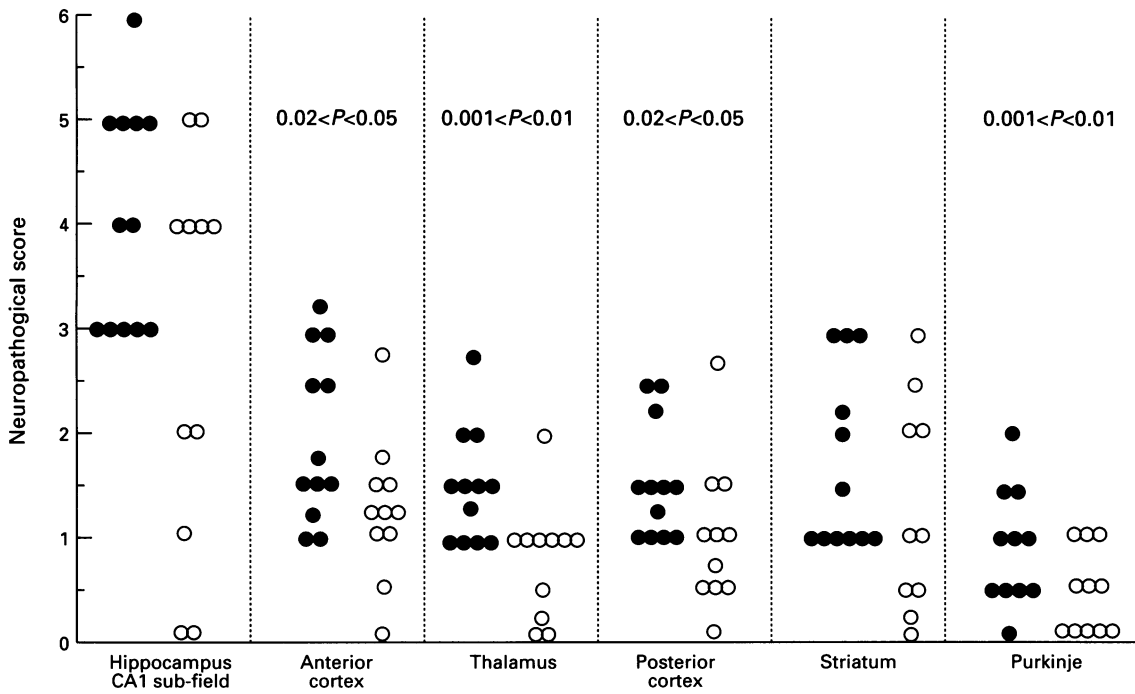

Figure 3 Data showing the scatterplot of the neuropathological scores, or level of damage, for a variety of brain areas for both vehicle (●) ($n=12$) and lifarizine (○) ($n=11$) treated rats following 2 vessel occlusion. The grading levels were: 0 = no damage; 1 = 0–10% damage; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100%; 6 = complete neuronal death, vacuolation, status spongiosus. Statistically significant reductions in neuronal damage following lifarizine treatment were evident in the anterior and posterior cortex, thalamus and purkinje cells of the cerebellar brain stem (Non-parametric Mann-Whitney analysis).

with the essential modification being manipulation of halothane concentration in individual animals to lower peripheral blood pressure and rapidly defeat the carotid (ictal) pressor response as soon as possible after occlusion of the carotid arteries. This caused the cortical EEG to quickly become isoelectric in all preparations and allowed more precise control of the 'real' injury time (about 1000 s of effective ischaemia) resulting from a 10 min occlusion. This was ultimately reflected by the achievement of a high-grade lesion where injury to several discrete types of vulnerable neurone populations could be quantified in relatively small groups of animals ($n=8-11$). We are fully aware that there may be concern over the possible adverse effect of high and varying concentrations of halothane on the final outcome, but it should be stated that the same anaesthetic conditions were present in both control and lifarizine-treated groups. It should also be noted that our modified model is probably more suitable for studies involving post-treatment, but care should be taken

when looking at pre-treatment studies due to the potential of interaction between the test substance and halothane. We were fully attentive to the problem of controlling brain temperature in this type of model and this was allowed for during surgery and recovery as discussed by Alps (1992).

Further refinement of the 4VO model involving the provision of ventilatory support prior to the induction of ischaemia allowed greater accuracy in determining the exact time of onset of EEG isoelectricity since previously the handling of animals to fit the face mask occurred at that time, and disturbed recording of the EEG signal for several seconds. Ideally, in this version of the 4VO model the period of effective ischaemia resulting from a standard 10 min period of occlusion was 1000–1200 s (Alps *et al.*, 1990). These modifications gave excellent results and enhanced the use of the model to a stage where it was possible to undertake dose-definition studies with lifarizine and show neuroprotection at i.a./i.p. doses of 2/10 $\mu g\ kg^{-1}$ to 100/500 $\mu g\ kg^{-1}$ (Alps *et al.*, 1990). Nevertheless, the modified 4VO model still required 2 stages of surgery and consideration was given to applying the principles learned in controlling the ictal response by anaesthetic manipulation alone to a 2VO version. Already it was being appreciated that the original 2VO model had advantages (Smith *et al.*, 1984; Ginsberg & Busto, 1989) over the original 4VO model of Pulsinelli & Brierley (1979) with which it was compared, being a one-stage procedure with good control of ventilation, having a lower mortality and still producing a high-grade forebrain ischaemia. The problem from our point of view was the need to use combined exsanguination and accompanying extra-pharmacological means of lowering peripheral blood pressure. The use of anaesthesia in a rodent model of global cerebral ischaemia does not necessarily invalidate the model (Jorgensen & Diemer, 1982; Schmidt-Kastner *et al.*, 1989; Freund *et al.*, 1990). A variety of alternative methods has been used to produce cerebral ischaemia in anaesthetized ventilated rats. These include raised intracranial pressure (Ross & Duhaime, 1989), cardiac arrest (Blomqvist & Wieloch, 1985), intrathoracic vascular occlusions (LeMay *et al.*, 1988), neck compression (Diemer & Siemkowicz, 1981), three vessel (Kameyama *et al.*, 1985) and even seven vessel (Shirane *et al.*, 1991) occlusion.

However, although the original versions of 2VO and 4VO models remain the most commonly used models of global cerebral ischaemia, the modifications to the 2VO model described in this paper offer the advantage of greater simplicity whilst retaining the stability of the measurable physiological parameters, blood gases, pH, and plasma glucose levels, and good control of blood pressure. During development of the modified 2VO model the effect of different periods of carotid artery occlusion was evaluated to establish whether the optimum time of effective ischaemia was still 10 min as with the modified 4VO model (Alps *et al.*, 1990). It was found that the quantification of neuronal damage in the anterior and posterior neocortices, thalamus, striatum and cerebellar brain stem were practically identical over this time span to that shown for 4VO, but damage to the hippocampal CA_1 cells was inconsistent to the point of being '*all or none*'. Increasing the period of arterial occlusion to 12 min increased the intensity of the ischaemic insult on these neurones and enhanced the reproducibility of neuropathological scores between separate groups of non drug-treated animals. As a result it can be seen in the present study that the duration of the isoelectric period for the EEG was extended nearly 1 min beyond that previously regarded as optimum in our revised 4VO model. Although the EEG recordings were made with surface electrodes, and therefore only give an indication of cortical activity during the insult period, the data from the [^{14}C]-iodoantipyrine study indicate that there is also a complete cessation of blood supply to the areas that would later be assessed in determining the neuroprotective efficacy of lifarizine. Previous global ischaemia studies have indicated that there was a small amount of flow in the forebrain structures during the ischaemic insult. However, our data show that there was no flow. This may be due to the fact that in our model the blood pressure dropped to as low as 30 mmHg at some points during the insult. It was necessary to extend the grading system from 0–4, as used by Alps *et al.* (1990) to 0–6 as described in this paper since more CA_1 ghost-cells appeared and the neutrophil showed evidence of vacuolation and status spongiosis. The extra component of reduced vascular perfusion had caused a more severe insult overall to the cerebral tissues but it was still 'therapeutically' retrievable in the model, as demonstrated by the use of an i.a/i.p. dose level of lifarizine previously shown to produce statistically significant protection to these structures in the 4VO model (Alps *et al.*, 1990). The basic problem in creating any animal model of disease is to ensure that one does not go too far and produce a level of damage which is actually untreatable. Models such as those described here are very delicately balanced and it is all too easy to produce a model which only describes the histopathology.

The neuroprotective profile of the diphenylpiperazine derivative, lifarizine (RS-87476; 1-[(2-(4-methylphenyl)-5-methyl)-1H-imidazol-4-yl-methyl]-4-diphenylmethyl-piperazine) has been shown to be neuroprotective in both global and focal models of cerebral ischaemia: the rat 4VO model (Alps *et al.*, 1990); gerbil unilateral carotid artery occlusion (Brown *et al.*, 1993); rat rose Bengal photochemical lesion (McBean *et al.*, 1995b); and mouse middle cerebral artery occlusion (Brown *et al.*, 1994). This neuroprotective efficacy led to the compound being tested in the cat permanent middle cerebral artery occlusion model by magnetic resonance imaging as well as histopathological techniques (Kucharczyk *et al.*, 1991). Greater than 70% protection was observed in cortical structures using i.v. infusion doses from 2 $\mu g\ kg^{-1}$ bolus +7 $\mu g\ kg^{-1}\ h^{-1}$ of the agent to 50 $\mu g\ kg^{-1}$ + 17.5 $\mu g\ kg^{-1}\ h^{-1}$ over 12 h. Thus, the neuroprotective effect in the cat at low parenteral doses substantiated the protective effect seen with lifarizine at similar doses in the rat 4VO model and low dose activity has now been confirmed in the rat 2VO model.

With regard to the mechanism of action of lifarizine, there is much evidence to suggest that the compound interacts with voltage-dependent sodium channels, since it has been shown to antagonize veratrine-induced contractures of chick cardiac myocytes (Patmore *et al.*, 1991), as well as inhibit the rat neocortical neuronal cell death, induced *in vitro* by veratridine, a site 2 sodium channel toxin (May *et al.*, 1995). Recent work has also shown the potent effects of lifarizine in inhibiting voltage-dependent sodium currents in N1E-115 mouse neuroblastoma cells (McGivern *et al.*, 1995). This ability of lifarizine to block selectively the inactivated sodium channels supports the hypothesis that lifarizine can reduce the hyperactivity of ischaemic neurones (excessively depolarized through a loss or reduction of metabolic support) without affecting the sodium channels in the non-ischaemic areas.

In conclusion, the modified rat 2VO model described here retains the previously stated advantages of the original 2VO models which are: it only requires a one-stage surgical procedure; it produces high-grade forebrain ischaemia; ventilation can be controlled to ensure normoxia and normocarbia; recirculation of the brain post-insult is easily achieved; it has a low experimental failure, and chronic survival studies can be performed when, in our opinion, the more appropriate neurological dysfunction related to memory and learning deficits can be assessed. It is a model of severe but controlled and therapeutically retrievable cerebral ischaemic damage which should prove suitable for screening potential neuroprotective agents.

References

ALPS, B.J. (1992). Drugs acting on calcium channels: potential treatment for ischaemic stroke. *Br. J. Clin. Pharmacol.*, **34**, 199–206.

ALPS, B.J., CALDER, C., WILSON, A.D. & PASCAL, J-C. (1990). Cerebral protection with a novel calcium blocker in rats. *Satellite Symposium to the ISIM 90 Congress 'Acute Care of Stroke'*, Sweden, Djuronaset, June 14–17.

ALPS, B.J. & HASS, W.K. (1987). The potential beneficial effect of nicardipine in a rat model of transient forebrain ischaemia. *Neurology*, **37**, 809–814.

BLOMQVIST, P. & WIELOCH, T. (1985). Ischaemic brain damage in rats following cardiac arrest using a long-term recovery model. *J. Cereb. Blood Flow Metab.*, **5**, 420–431.

BROWN, A. & BRIERLEY, J. (1968). The nature, distribution and earliest stages of anoxic-ischaemic nerve cell damage in the rat brain as defined by the optical microscope. *Br. J. Exp. Pathol.*, **49**, 87–106.

BROWN, C.M., CALDER, C., ALPS, B.J. & SPEDDING, M. (1993). The effect of lifarizine (RS-87476), a novel sodium and calcium channel modulator, on ischaemic dopamine depletion in the corpus striatum of the gerbil. *Br. J. Pharmacol.*, **109**, 175–177.

BROWN, C.M., SMALL, C., CALDER, C. & PATMORE, L. (1994). Neuroprotective properties of lifarizine (RS-87476) in a mouse model of focal ischaemia. *Can. J. Physiol. Pharmacol.*, **72** (Suppl. 1), 435.

DIEMER, N.H. & SIEMKOWICZ, E. (1981). Regional neuronal damage after cerebral ischaemia in the normo- and hypoglycaemic rat. *Neuropath. Appl. Neurobiol.*, **7**, 217–227.

EKLOF, B. & SIESJO, B. (1972). The effect of bilateral carotid artery ligation upon the blood flow and energy state of the rat brain. *Acta Physiol. Scand.*, **86**, 155–165.

EKLOF, B. & SIESJO, B. (1973). Cerebral blood flow in ischaemia caused by carotid artery ligation in the rat. *Acta Physiol. Scand.*, **87**, 69–77.

FREUND, T.F., BUSZAKI, G., LEON, A. & SOMOGYI, P. (1990). Hippocampal cell death following ischaemia: effects of brain temperature and anaesthesia. *Exp. Neurol.*, **108**, 251–260.

GINSBERG, M.D. & BUSTO, R. (1989). Rodent models of cerebral ischaemia. *Stroke*, **20**, 1627–1642.

JORGENSEN, M.B. & DIEMER, N.H. (1982). Selective neuronal loss after cerebral ischaemia in the rat. Possible role of transmitter glutamate. *Acta Neurol. Scand.*, **66**, 536–546.

KAMEYAMA, M., SUZUKI, J., SHIRANE, R. & OGAWA, A. (1985). A new model of bilateral hemispheric ischaemia in the rat - three vessel occlusion model. *Stroke*, **16**, 489–493.

KUCHARCZYK, J., MINTOROVITCH, J., MOSELEY, M.E., ASGARI, H.S., SEVICK, R.J., DERUGIN, N. & NORMAN, D. (1991). Ischaemic brain damage: reduction by sodium-calcium ion channel modulator RS-87476. *Radiology*, **179**, 221–227.

LE MAY, D.R., GEHUA, L., ZELENOCK, G.B. & D'ALECY, L.G. (1988). Insulin administration protects neurologic function in cerebral ischaemia in rats. *Stroke*, **19**, 1411–1419.

MCBEAN, D.E., WINTERS, V., WILSON, A.D., OSWALD, C.B. & ARMSTRONG, J.M. (1995a). Neuroprotective efficacy of the ion channel modulator lifarizine (RS-87476) in a rodent model of global cerebral ischaemia. *Br. J. Pharmacol.*, **114**, 331P.

MCBEAN, D.E., WINTERS, V., WILSON, A.D., OSWALD, C.B. & ARMSTRONG, J.M. (1995b). The ion channel modulator lifarizine (RS-87476) reduces the size of the infarct produced in the rat by the rose Bengal photochemical focal model of cerebral ischaemia. *Br. J. Pharmacol.*, **114**, 332P.

MCGIVERN, J.G., PATMORE, L. & SHERIDAN, R.D. (1995). Actions of the novel neuroprotective agent, lifarizine (RS-87476), on voltage-dependent sodium currents in the neuroblastoma cell line. *Br. J. Pharmacol.*, **114**, 1738–1744.

MAY, G.R., ROWAND, W.S., MCCORMACK, J.G. & SHERIDAN, R.D. (1995). Neuroprotective profile of lifarizine (RS-87476) in rat cerebrocortical neurones in culture. *Br. J. Pharmacol.*, **114**, 1365–1370.

PATMORE, L., DUNCAN, G.P., KENNY, B.A. & SPEDDING, M. (1991). RS-87476, a novel neuroprotective agent, inhibits veratrine-induced calcium overload in embryonic chick cardiac myocytes. *Br. J. Pharmacol.*, **104**, 175P.

PULSINELLI, W.A. & BRIERLEY, J.B. (1979). A new model of bilateral hemispheric ischaemia in the unanaesthetized rat. *Stroke*, **10**, 267–272.

ROSS, D.T. & DUHAIME, A-C. (1989). Degeneration of neurons in the thalamic reticular formation nucleus following transient ischaemia due to raised intracranial pressure: excitotoxic degeneration mediated via non-NMDA receptors? *Brain Res.*, **501**, 129–143.

SAKURADA, O., KENNEDY, C., JEHLE, J., BROWN, J.D., CARBIN, G.L. & SOKOLOFF, L. (1978). Measurement of cerebral blood flow with iodo-[^{14}C]-antipyrine. *Am. J. Physiol.*, **234**, H59–H66.

SCHMIDT-KASTNER, R., PASCHEN, W., GROSSE OPHOFF, B. & HOSSMANN, K.A. (1989). A modified four-vessel occlusion model for inducing incomplete forebrain ischaemia in rats. *Stroke*, **20**, 938–946.

SHIRANE, R., SHIMIZIN, H., KAMEYAMA, M. & WEINSTEIN, P.R. (1991). A new method for producing temporary complete cerebral ischaemia in rats. *J. Cereb. Blood Flow Metab.*, **11**, 949–956.

SMITH, M-L., BENDEK, G., DAHLGREN, N., ROSEN, I., WIELOCH, T. & SIESJO, B.K. (1984). Models for studying long-term recovery following forebrain ischaemia in the rat. A 2 vessel occlusion model. *Acta Neurol. Scand.*, **69**, 385–401.

(Received May 19, 1995
Revised July 25, 1995
Accepted August 23, 1995)