Lack of effect of L-687,414 ((+)-*cis*-4-methyl-HA-966), an NMDA receptor antagonist acting at the glycine site, on cerebral glucose metabolism and cortical neuronal morphology

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1 N-methyl-D-aspartate (NMDA) receptor ion channel antagonists have been reported to cause pronounced increases in cerebral glucose metabolism (CMR_{glc}) and transient reversible vacuolation within pyramidal cortical neurones. The present studies examined in rats the effects of the NMDA receptor antagonist, L-687,414 (R-(+)-*cis*-4-methyl-3-amino-1-hydroxypyrolid-2-one; (+)-*cis*-4-methyl-HA-966) on regional CMR_{glc} and cortical neuronal morphology.

2 L-687,414 was given as a steady state intravenous infusion for 4 h in a neuroprotective dose regime of 17.5 mg free base kg^{-1} bolus followed by 225 $\mu g\ kg^{-1}\ min^{-1}$ ($n = 8$) or at the higher dose rate of 35 mg kg^{-1} bolus followed by 440 $\mu g\ kg^{-1}\ min^{-1}$ ($n = 10$). Data were compared to a parallel series of experiments in rats given the NMDA receptor ion channel antagonist, dizocilpine for 4 h in the optimum intravenous neuroprotective dose-regime of 0.12 mg kg^{-1} bolus followed by 1.8 $\mu g\ kg^{-1}\ min^{-1}$ ($n = 8$) or at the higher dose rate of 0.4 mg kg^{-1} bolus followed by 6 $\mu g\ kg^{-1}\ min^{-1}$ ($n = 4$; morphology only studied). A saline-infused group of rats ($n = 8$) were used as controls.

3 CMR_{glc} was studied by use of [^{14}C]-2-deoxyglucose and autoradiography ($n = 4$ each group) whilst plasma drug levels were in a steady state during the final 45 min of the 4 h drug infusion. Effects on cortical neuronal morphology were assessed at the end of the 4 h infusion period using light microscopic techniques ($n = 4$ –6 each group).

4 The results showed a selective activation of limbic CMR_{glc} by dizocilpine at optimal neuroprotective dose levels and showed that this dose was at the threshold for the neuronal vacuolation response as 1 of 4 rats showed morphological changes in the pyramidal neurones in the posterior cingulate and retrosplenial cortices. At the higher dose rate of dizocilpine, all 4 animals showed extensive morphological changes in these cortical neurones. In contrast, L-687,414 did not increase limbic CMR_{glc} nor evoke vacuolation when given in the neuroprotective dose-regime or at the higher dosage rate.

5 The findings of the present study suggest that neuroprotection mediated through the NMDA receptor complex can be achieved without changes in CMR_{glc} or cortical neuronal morphology by antagonism at the glycine modulatory site.

Keywords: L-687,414; dizocilpine; NMDA glycine; partial agonist; cerebral glucose metabolism; cortical neuronal morphology

Introduction

Overactivation of N-methyl-D-aspartate (NMDA) receptors by excitatory amino acids released during cerebral ischaemia is thought to result in an excessive calcium influx into neurones that causes cell death (Choi, 1990; Benveniste, 1991). NMDA receptor antagonists have been shown to be neuroprotective in various experimental models of focal ischaemia (McCulloch, 1991) and to protect against excitotoxin-induced CNS nerve cell loss (Foster *et al.*, 1988; 1990). Antagonism of the excitotoxic effects associated with excessive NMDA receptor activation can be achieved by actions at several distinct sites within the NMDA receptor complex (Wong & Kemp, 1991). NMDA antagonists may act within the ion channel controlled by the receptor producing a use-dependent non-competitive blockade, competitively at the glutamate recognition site or at allosteric modulatory sites on the receptor complex where glycine and polyamines are thought to act to enhance NMDA receptor function.

The administration of some non-competitive NMDA receptor channel antagonists (phencyclidine and the related compounds dizocilpine (MK-801) and ketamine) has been shown to increase cerebral glucose metabolism (CMR_{glc}) particularly within the limbic system (Kurumaji *et al.*, 1989; Nehls *et al.*, 1990). The same NMDA receptor channel antagonists have also been shown to induce transient reversi-

ble vacuolation in pyramidal neurones in the posterior cingulate and retrosplenial cortices in the rat (Olney *et al.*, 1989), areas of the CNS coincident anatomically with regions in which glucose hypermetabolism is observed (Allen & Iversen, 1990).

L-687,414 (R-(+)-*cis*-4-methyl-HA-966) is an NMDA antagonist that acts at the glycine modulatory site of the NMDA receptor complex, but at high concentrations shows characteristics typical of a low efficacy partial agonist (Kemp *et al.*, 1991). L-687,414 has higher affinity and slightly lower intrinsic activity at the glycine site than HA-966 (Foster *et al.*, 1991). L-687,414 has been shown to be active in anticonvulsant tests in rodents (Saywell *et al.*, 1991) and primates (Smith & Meldrum, 1992) and to give significant neuroprotection when given post-occlusion in the rat middle cerebral artery occlusion (MCAO) model of focal cerebral ischaemia (Gill *et al.*, 1991a).

The objectives of the present studies were to examine the effects of neuroprotective dose regimes of L-687,414 on CMR_{glc} and neuronal morphology in comparison with the NMDA receptor channel blocker dizocilpine, the most potent neuroprotective agent yet described (Gill *et al.*, 1991b). The study design mimicked that of experimental studies of focal ischaemia in rats and attempted to simulate some of the conditions that may occur in the clinic where neuroprotective agents might be given by bolus intravenous injection follow-

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ed by slow intravenous infusion to sustain plasma concentrations for prolonged periods of time. A preliminary account of part of these studies was given at the XVth International Symposium on Cerebral Blood Flow and Metabolism (Hargreaves *et al.*, 1991).

Methods

Male Sprague-Dawley rats (approximately 260 g body weight) were anaesthetized with isoflurane and the tail artery and lateral tail veins cannulated for blood sampling and the administration of test compounds, radiolabelled tracer or 0.9% saline vehicle. After surgery the animals were placed in a quiet room in open restraining cages (that allow movement but prevent turning to gain access to the vascular cannulae) and allowed to recover for 2 h before dosing.

After recovery the rats were given either L-687,414 or MK-801 intravenously. Groups of rats were given L-687,414 as a 17.5 mg kg⁻¹ bolus followed by infusion at 225 µg kg⁻¹ min⁻¹ ($n = 8$: the neuroprotective dose regime in the rat MCAO model of focal ischaemia (Gill *et al.*, 1991a)) or at the higher dose rate of 35 mg kg⁻¹ bolus followed by 440 µg kg⁻¹ min⁻¹ ($n = 10$). For comparison, groups of rats were given dizocilpine intravenously as a 0.12 mg kg⁻¹ bolus followed by infusion at 1.8 µg kg⁻¹ min⁻¹ ($n = 8$: the optimal neuroprotection regime in the rat MCAO model of focal ischaemia (Gill *et al.*, 1991b)) or the higher dose rate of 0.4 mg kg⁻¹ bolus followed by 6 µg kg⁻¹ min⁻¹ ($n = 4$). As control, a group of rats were given saline (vehicle) as a bolus 1.0 ml kg⁻¹ followed by infusion at 4.27 µl kg⁻¹ min⁻¹, giving an identical dose volume to the animals receiving L-687,414 or dizocilpine. All infusions lasted for 4 h. From each group, 4 rats were used to examine effects on cerebral glucose metabolism and the remainder to study neuronal morphology. In the high dose dizocilpine group, only neuronal morphology was examined. Regional cerebral glucose metabolism was measured during the final 45 min of a 4 h drug infusion and effects on neuronal morphology studied at 4 h, at the end of dosing.

Cardiovascular effects

The blood pressure effects of L-687,414 given in the dosing regimes used in this study were monitored from the tail artery catheter in animals used for neuronal morphology studies. Heart rate was derived from the blood pressure signal. The blood pressure and heart rate effects of dizocilpine were recorded in separate groups of animals ($n = 4$ in each) that were treated identically to the two dizocilpine dose groups used in the present studies.

Plasma drug levels

Plasma levels of L-687,414 in rats from both the low and high dose groups used to study neuronal morphology were measured by high performance liquid chromatography (h.p.l.c.) using fluorescence detection after solid phase extraction and pre-column derivatisation with FMOC chloride. Plasma dizocilpine levels resulting from the present dosing regimes have been reported previously (Gill *et al.*, 1991b). Plasma profiles representative of rats in both of the dizocilpine dosed groups in the present studies were determined by radioimmunoassay (Hichens *et al.*, 1990).

Regional cerebral glucose metabolism ($rCMR_{glc}$)

Measurements of glucose metabolism were limited to a series of structures within the limbic and auditory systems (cerebellar vermis as negative control area) since changes selectively within these structures were shown previously to be characteristic following the administration of NMDA antagonists (Nehls *et al.*, 1988). Autoradiographic studies of

$rCMR_{glc}$ were made whilst the plasma levels of L-687,414 and dizocilpine were in a steady state. $rCMR_{glc}$ was measured using the 2-deoxyglucose (2-DG) method essentially as described previously by Sokoloff *et al.* (1977). At 45 min before the end of the drug infusion schedules, approximately 125 µCi kg⁻¹ [¹⁴C]-2-DG was given intravenously in a dose volume of 1 ml kg⁻¹ body weight. A blood sample was taken before the administration of [¹⁴C]-2-DG and 14 small blood samples were taken afterwards over 45 min. Plasma fractions were separated from the blood samples and assayed for radioactivity and glucose content. At 45 min after administration of label, rats were killed by rapid i.v. injection of pentobarbitone Na (200 mg ml⁻¹) and decapitated. The brain was removed and frozen in isopentane at -45°C. The frozen brain was sectioned (20 µm) in a cryostat and 3 of every 10 sections mounted on coverslips, dried at 60°C and placed in apposition to Hyperfilm along with precalibrated ¹⁴C standards. Local tissue levels of ¹⁴C were determined by quantitative densitometry. For each region of the brain analysed, 12 bilateral density readings were made on a series of six sections in which the structure was clearly identifiable. Regional cerebral glucose metabolism (CMR_{glc}) was calculated using the standard operational equation from tissue ¹⁴C, plasma ¹⁴C and plasma glucose levels (Sokoloff *et al.*, 1977).

Neuronal morphology

At the end of the 4 h drug treatment regime the animals were anaesthetized with 60 mg kg⁻¹ pentobarbitone Na i.p. and perfused transcardially with ice-cold heparinised saline at 100 mmHg pressure. Following exsanguination with saline the perfusion was changed to freshly prepared 1% paraformaldehyde/1.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) at pH 7.4. Once fixed the brain was removed from the skull and allowed to stand in cold fixative for 1 h. The brain was then sliced into 1 mm transverse sections and sampled for the areas of interest (posterior cingulate and retrosplenial cortices) which were kept in fixative at 4°C overnight.

The samples were washed three times with 0.1 M PBS before post-fixation in 1 ml of 1% osmium tetroxide at 4°C overnight. The samples were again washed three times with PBS before dehydration in alcohol, and infiltration with araldite. The samples were embedded and polymerised at 60°C overnight before curing for 12 h and sectioning. Tissue sections (1 µm) were stained with methylene blue/Azure II. Neuronal morphology was assessed by examining the entire area of representative sections through each brain area by light microscopy at ×1000 magnification. Sections were examined by an independent observer who was unaware of the treatments given.

Materials

2-Deoxy-D-[1-¹⁴C]-glucose was purchased from Amersham (CFA 728; 50–60 mCi mmol⁻¹) together with autoradiographic ¹⁴C microscopes (31–883 nCi g⁻¹; RPA504L). Autoradiographic film was Hyperfilm β_{max} from Amersham. All other compounds were obtained from Sigma Chemical Co or Fisons Scientific.

Results

Representative plasma profiles for L-687,414 and dizocilpine are shown in Figures 1 and 2. The mean plasma concentrations of L-687,414 over the 4 h dosing period were 19 ± 1 µg ml⁻¹ in the low dose group and 54 ± 3 µg ml⁻¹ in the high dose group. Similarly, the mean plasma concentrations of dizocilpine were 22 ± 6 ng ml⁻¹ and 73 ± 8 ng ml⁻¹.

Figure 3 shows that dizocilpine administration caused an immediate dose-related rise in blood pressure that was sustained throughout the drug infusion period. In contrast, L-

687,414 did not affect blood pressure at either the low or high dose level. Dizocilpine also caused a concomitant increase in heart rate (+18%) but no effect was observed with L-687,414 (Figure 4).

Plasma glucose levels at the start of 2-deoxyglucose experiments, 3.25 h after the start of drug or vehicle infusions, were 6.6 ± 0.6 mM (saline), 8.0 ± 0.4 mM (dizocilpine), 7.2 ± 0.4 mM (low dose L-687,414) and 7.8 ± 0.6 mM (high dose L-687,414). The dizocilpine group showed behavioural changes (head weaving, followed by reduced spontaneous activity) but these were not observed in animals given L-687,414.

The effects of L-687,414 and dizocilpine on regional CMR_{glc} are given in Table 1. Dizocilpine administration caused pronounced increases in CMR_{glc} in the hippocampus and entorhinal cortex and throughout the component regions of the Papez circuit (see McCulloch & Iversen, 1991). In contrast, CMR_{glc} was moderately decreased in auditory structures. Unlike dizocilpine, L-687,414 did not evoke increases

in limbic CMR_{glc} at either dose level tested. Glucose use appeared slightly depressed at the higher dose of L-687,414 particularly in auditory regions. The studies of neuronal morphology (Figure 5) detected neuronal vacuolation in the pyramidal neurones of the posterior cingulate and retrosplenial cortices of one of 4 rats given dizocilpine at the lower dose level. At the high dose of dizocilpine, all four animals showed extensive neuronal morphological changes (Figure 5c). The neurones appeared swollen with numerous vacuoles evident throughout the cytoplasmic compartment. The changes were most apparent in medium and large sized neurones in layer III of the cortices with small neurones in layer II being unaffected. The glycine site antagonist L-687,414 did not evoke vacuolation at the low or high dose levels (Figure 5b). All neurones showed normal cytoplasmic morphology (dense basophilic even-staining) and were indistinguishable from those of control saline-infused animals (Figure 5a).

Figure 1 Plasma levels of L-687,414 during intravenous bolus and infusion: (○) 17.5 mg kg^{-1} bolus followed by $225 \text{ µg kg}^{-1} \text{ min}^{-1}$, mean plasma level is 19 µg ml^{-1} ; (●) 35 mg kg^{-1} bolus followed by $440 \text{ µg kg}^{-1} \text{ min}^{-1}$, mean plasma level is 54 µg ml^{-1} . Points are mean $\pm$ s.e.mean. $n = 4$ or 6 .

Figure 2 Plasma levels of dizocilpine during intravenous bolus and infusion: (□) 0.12 mg kg^{-1} bolus followed by $1.8 \text{ µg kg}^{-1} \text{ min}^{-1}$, mean plasma level is 22 ng ml^{-1} ; (■) 0.4 mg kg^{-1} bolus followed by $6 \text{ µg kg}^{-1} \text{ min}^{-1}$, mean plasma level is 73 ng ml^{-1} . Points are mean $\pm$ s.e.mean. $n = 3$.

Figure 3 Changes in mean arterial blood pressure during the intravenous bolus injection and infusion of (■) dizocilpine, 0.4 mg kg^{-1} bolus + $6 \text{ µg kg}^{-1} \text{ min}^{-1}$ (104 ± 4); (□) dizocilpine, 0.12 mg kg^{-1} bolus + $1.8 \text{ µg kg}^{-1} \text{ min}^{-1}$ (110 ± 3); (●) L-687,414, 35 mg kg^{-1} bolus + $440 \text{ µg kg}^{-1} \text{ min}^{-1}$ (102 ± 2); (○) L-687,414 17.5 mg kg^{-1} + $225 \text{ µg kg}^{-1} \text{ min}^{-1}$ (104 ± 1); (Δ) saline, 1.0 ml kg^{-1} + $4.27 \text{ µg kg}^{-1} \text{ min}^{-1}$ (118 ± 2). Starting blood pressures (mmHg $\pm$ s.e.mean) are given in parentheses. Data points are mean $\pm$ s.e.mean. $n = 4$ or 6 .

Figure 4 Changes in heart rate during the intravenous bolus injection and infusion of (■) dizocilpine, 0.4 mg kg^{-1} bolus + $6 \text{ µg kg}^{-1} \text{ min}^{-1}$ (416 ± 26); (□) dizocilpine, 0.12 mg kg^{-1} bolus + $1.8 \text{ µg kg}^{-1} \text{ min}^{-1}$ (425 ± 24); (●) L-687,414, 35 mg kg^{-1} bolus + $440 \text{ µg kg}^{-1} \text{ min}^{-1}$ (433 ± 13); (○) L-687,414 17.5 mg kg^{-1} + $225 \text{ µg kg}^{-1} \text{ min}^{-1}$ (370 ± 22). (Δ) Saline, 1.0 ml kg^{-1} + $4.27 \text{ µg kg}^{-1} \text{ min}^{-1}$ (460 ± 22). Starting heart rates (beats min^{-1} $\pm$ s.e.mean) are given in parentheses. Data points are mean $\pm$ s.e.mean. $n = 4$ or 6 .

Figure 5 Comparison of the effects of saline ($1.0 \text{ ml kg}^{-1} + 4.67 \mu\text{l kg}^{-1} \text{ min}^{-1}$), L-687,414 ($35 \text{ mg kg}^{-1} \text{ bolus} + 440 \mu\text{g kg}^{-1} \text{ min}^{-1}$) and dizocilpine ($0.4 \text{ mg kg}^{-1} \text{ bolus} + 6 \mu\text{g kg}^{-1} \text{ min}^{-1}$) on neuronal morphology in the rat posterior cingulate cortex. In rats given saline (a) and L-687,414 (b) the cytoplasm of medium and large pyramidal neurones is uniformly well stained whereas in rats given dizocilpine (c) it is swollen and pale and shows extensive vacuolation. Bar = $50 \mu\text{m}$.

Table 1 Effects of L-687,414 and dizocilpine on regional cerebral glucose metabolism

Region	Saline	Dizocilpine ^a	L-687,414 ^b	L-687,414 ^c
<i>Limbic system</i>				
Hippocampal molecular layer (P)	62 ± 4	110 ± 10**	57 ± 7	52 ± 6
Dentate gyrus	54 ± 3	74 ± 4*	55 ± 5	45 ± 5
Anteroventral thalamic nucleus (P)	90 ± 4	228 ± 39**	85 ± 9	76 ± 12
Posterior cingulate cortex (P)	86 ± 4	179 ± 14**	80 ± 5	57 ± 6
Retrosplenial cortex (P)	68 ± 4	159 ± 22**	80 ± 8	58 ± 6
Entorhinal cortex (P)	48 ± 3	90 ± 8**	52 ± 9	42 ± 6
<i>Auditory system</i>				
Auditory cortex	120 ± 7	100 ± 6	116 ± 7	63 ± 6**
Medial geniculate body	100 ± 10	67 ± 3**	82 ± 4	46 ± 5**
Inferior colliculus	143 ± 18	106 ± 10	143 ± 7	79 ± 5**
Superior olivary nucleus	122 ± 21	108 ± 6	122 ± 7	107 ± 8
<i>Other structures</i>				
Cerebellar vermis	42 ± 3	50 ± 7	41 ± 3	38 ± 4
Caudate	96 ± 8	146 ± 19*	91 ± 3	79 ± 13

^aDizocilpine 0.12 mg kg⁻¹ followed by infusion of 1.8 µg kg⁻¹ min⁻¹;^bL-687,414; 17.5 mg kg⁻¹ followed by infusion of 225 µg kg⁻¹ min⁻¹;^cL-687,414; 35 mg kg⁻¹ followed by infusion of 440 µg kg⁻¹ min⁻¹

(P) Components of the limbic Papez circuit.

Data are CMR_{glc} (µmol 100 g⁻¹ min⁻¹) ± s.e.mean**P* < 0.05; ***P* < 0.01 ANOVA and Dunnett's multiple comparison with respect to saline dosed controls

Discussion

The intravenous infusion of dizocilpine at neuroprotective dose levels was associated with dose-related sustained systemic hypertension and tachycardia in conscious rats. Similar effects have been reported with bolus intravenous doses of ketamine (Traber *et al.*, 1971; White *et al.*, 1982) and with dizocilpine itself (Lewis *et al.*, 1989). These cardiovascular effects are thought to reflect centrally mediated increases in sympathetic nerve activity. Sympathoexcitatory effects were not observed with L-687,414 such that the cardiovascular profile was similar to the saline controls.

Plasma levels of the lower doses of L-687,414 and dizocilpine in the present experiments (19 µg ml⁻¹ and 22 ng ml⁻¹ respectively) were similar to those (25 µg ml⁻¹ and 19 ng ml⁻¹ respectively) achieved with the same dosing regimes in the rat MCAO studies of focal ischaemia that were conducted in parallel in our laboratories (Gill *et al.*, 1991a,b). Plasma levels produced by the higher dose levels of L-687,414 (54 µg ml⁻¹) and dizocilpine (73 ng ml⁻¹) exceeded those required for neuroprotection. The slightly lower plasma levels in conscious rats after infusion of L-687,414 in the neuroprotective dose regime probably reflects slightly faster plasma clearance than occurs in the isoflurane anaesthetized animals used for MCAO studies.

The autoradiographic studies of regional CMR_{glc} were made with the plasma drug levels in the steady state, a condition of the 2-deoxyglucose methodology that is rarely met in studies of CMR_{glc} after intravenous bolus injection of test compounds. The results showed that dizocilpine caused pronounced activation of CMR_{glc} in the glutamatergic perforant pathway that projects from the entorhinal cortex and terminates in the dentate gyrus and hippocampus. It has been hypothesized, on the basis that the hippocampus contains a very high density of NMDA receptors, that blockade in this brain region evokes increased input cell firing in an attempt to overcome the block (McCulloch & Iversen, 1991). Since NMDA antagonism with receptor ion channel blocking agents is both non-competitive and use-dependent, this increased firing leads to an intensification of the blockade and increased CMR_{glc} around the limbic Papez circuit (see Table 1).

If this hypothesis is correct then it is clear that the level of blockade that can be achieved with L-687,414 is insufficient to evoke this response despite being effective against focal ischaemia following MCAO. Whatever the mechanism, the present findings show that neuroprotection with NMDA antagonists can be achieved without limbic neuronal activa-

tion. It is interesting to note that L-687,414 given to conscious rats at 100 mg free base kg⁻¹ intravenously did not stimulate dopamine metabolism (measured as the DOPAC + HVA/dopamine ratio) in the nucleus accumbens within the limbic system or in the striatum. In contrast, dizocilpine at 0.2 mg kg⁻¹ intravenously produced a large increase in dopamine turnover in the accumbens, indicative of greatly increased neuronal activity (Hutson, personal communication).

Olney *et al.* (1989) first reported that acute administration of several non-competitive NMDA receptor channel antagonists (phencyclidine, tiletamine and dizocilpine) induced dose-dependent reversible swelling and vacuolation in pyramidal neurones in the rat posterior cingulate and retrosplenial cortices. It has been suggested that there may be age-related changes in the susceptibility of rats to the cortical morphological effects of the NMDA receptor ion channel blockers with responsivity first being observed at 30 days (Sharp *et al.*, 1992) increasing to three months of age (Farber *et al.*, 1992). In the present studies the rats used were approximately 12 weeks old and were age-matched across treatment groups. These rats were sensitive to the metabolic and morphological effects of dizocilpine and thus provided a suitable positive control. There is as yet no published evidence for age-related changes in sensitivity with other classes of NMDA receptor antagonists. Chronic administration of dizocilpine (1 mg kg⁻¹ day⁻¹ for 14 days) to mature Sprague-Dawley rats (>12 weeks of age) was not associated with neuronal cell loss, suggesting that the vacuolation seen shortly after acute dosing is not a prelude to significant long-term neuronal damage. Only after very high acute doses (5 mg kg⁻¹ dizocilpine, s.c.) were a few neurones (<0.5%) found to be irreversibly damaged when CNS tissues were examined 48 h after dosing (Allen & Iversen, 1990; Allen *et al.*, 1991).

The present studies indicate that the doses of dizocilpine required for optimal neuroprotection (Gill *et al.*, 1991b) are at the threshold of those for inducing changes in neuronal morphology since one of four rats showed a vacuolation response. Thus, there is little margin between wanted and unwanted effects with dizocilpine. L-687,414 did not alter cortical neuronal morphology when given to conscious rats in the neuroprotective dose regime (Gill *et al.*, 1991a) or at a higher rate that produced two to threefold higher plasma levels, indicating that it may have a greater therapeutic window. This estimate of the potential therapeutic window with L-687,414 is likely to be a lower limit as no cardiovascular sympathoexcitation, increased CMR_{glc} nor changes in cortical neuronal morphology were detected at either of the plasma

levels tested. It should be noted that the present studies were not designed to investigate the neurotoxic profile of L-687,414 but to establish, by direct comparison with rat MCAO experiments conducted in parallel in our laboratories (Gill *et al.*, 1991a,b), that there is a clear window between neurotoxicity and neuroprotection with L-687,414 that is absent with the NMDA receptor ion channel blocker, dizocilpine. Comparison with another neuroprotection study in which the plasma levels of L-687,414 and dizocilpine were measured suggests that the window for L-687,414 is at least 18 whilst that for dizocilpine is <2 (Rigby *et al.*, 1992). If the total dose of L-687,414 administered over 4 h in the present experiments is used for such comparisons, then the window for L-687,414 appears much larger still.

Scatton and colleagues have shown that SL 82.0715, an NMDA antagonist acting at the polyamine site, does not evoke increased CMR_{glc} nor induce neuromorphological changes in rat CNS (Scatton *et al.*, 1991; Duval *et al.*, 1992). These results, together with those from the present studies, suggest that the potential therapeutic window for NMDA receptor antagonists may depend upon the specific site within the NMDA receptor complex at which the compound acts to reduce activation. This view is supported by our observation that competitive NMDA receptor antagonists acting at the glutamate recognition site are capable of increasing limbic CMR_{glc} and producing cortical neuronal vacuolation but, nevertheless, appear also to have a greater potential therapeutic window than dizocilpine (Hargreaves *et al.*, 1993). The mechanism of vacuolation in cortical neurones is unclear. The anatomical coincidence of morphological changes and increased CMR_{glc} has implicated hypermetabolism in the dizocilpine vacuolation response and several observations support this view. Parallels have been drawn between the morphological changes in neurones after periods of hypermetabolism associated with seizure activity (McCulloch & Iversen, 1991) suggesting that vacuolation may be linked in some way to the CMR_{glc} response. The present studies and those of Duval *et al.* (1992) have shown that L-687,414 and SL 82.0715 do not increase CMR_{glc} in rat brain and do not induce vacuolation in the posterior cingulate and retrosplenial cortical neurones.

The cortical morphological effects of NMDA receptor ion channel blockers have been shown (Olney *et al.*, 1991) to be antagonized by muscarinic antagonists and drugs acting at GABAergic sites (barbiturates, diazepam) indicating an involvement of pathways with these receptor types in the vacuolation response. Sharp *et al.* (1991, 1992) have suggested that drug-induced vacuolar changes and altered intracellular proteins in cortical neurones then act as a stimulus for the later expression of heat shock protein. Importantly, heat shock protein expression appears to be primarily in injured neurones that are destined to survive (Lindsberg *et al.*, 1991; Sharp *et al.*, 1991; Welsh *et al.*, 1991). Thus, neuronal vacuolation can be viewed as an early marker of drug-induced cellular stress. Production of heat shock protein has been shown to be inhibited by agents that prevent vacuolation (Olney *et al.*, 1991) supporting the idea that their pathogenesis is linked to a common mechanism. Olney's findings suggest a specific involvement of cholinergic muscarinic pathways in the response to the NMDA receptor ion channel blockers (Olney *et al.*, 1991). Indeed, the changes in the pattern of coupling of regional cerebral blood flow and glucose metabolism after dizocilpine (Nehls *et al.*, 1990; McCulloch & Iversen, 1991) resemble those that result from stimulation of the fastigial nucleus that is thought to activate ascending cholinergic pathways (Iadecola *et al.*, 1983; Nakai *et al.*, 1983). It is noteworthy that dizocilpine caused profound hyperaemia, in excess of the increase in cerebral blood flow expected as a result of increased metabolic activity in

the cingulate cortex (see McCulloch & Iversen, 1991). Heightened blood flow may remove lactate accumulated as a result of the maintenance of intracellular ionic homeostasis by glycolysis during the intense activation of cingulate neurones. Interestingly, following ketamine administration, cerebral blood flow was also found to increase in excess of metabolic demand within the cingulate cortex (Cavazzuti *et al.*, 1987).

Recent studies on the protective effects of haloperidol and rimcazole against heat shock protein induction by phencyclidine and dizocilpine support the involvement of multiple receptor subtypes in the cortical morphological response (Sharp *et al.*, 1992). However, doses of haloperidol in excess of 5 mg kg^{-1} may cause generalized CNS depression. This observation together with those on the protective effects of pretreatment with gaseous anaesthetics (Kurumaji & McCulloch, 1989; Hargreaves & Rigby unpublished observations) indicate that non-specific depression of CNS activity may also be involved in the action of the compounds that antagonize the cortical morphological effects of NMDA receptor ion channel blockers, and could be a means of controlling their potential adverse side-effects. It should be noted, however, that when dizocilpine is given before halothane anaesthesia then protection is not seen (Olney *et al.*, 1991).

It remains to be determined whether the apparent differences in therapeutic window for NMDA receptor antagonists acting at different sites within the receptor complex are due to true differences in side-effect liability or reflect a greater ability to 'fine-tune' the level of blockade so that the neuroprotective and morphological effects can be divorced. Clearly the actions of competitive antagonists acting at the glycine and glutamate recognition sites will be critically influenced by the prevailing concentrations of the endogenous ligands unlike the uncompetitive ion channel blocking agents. Since endogenous brain glycine concentrations are thought to be near saturating for the NMDA glycine site, it may be very difficult with a competitive antagonist acting at this site to achieve the same levels of blockade that can be attained with a channel blocking agent such as dizocilpine. Interpretation of the actions of the glycine site antagonist L-687,414 are complicated somewhat by its partial agonist properties (Kemp *et al.*, 1991). On voltage clamped cultured cortical neurones, L-687,414 antagonized glycine and NMDA responses competitively but reached a plateau of effect at 90% inhibition and showed weak agonist effects (6% of the maximum response to glycine) at high concentrations. Thus, it appears likely that blockade of the NMDA receptor, equivalent to that possible with an NMDA receptor channel blocker, cannot be achieved with L-687,414 and it may be this that restricts its side-effect potential. Further examination of site-related differences in the potential therapeutic window for NMDA antagonists awaits the testing of a brain penetrant, glycine site full antagonist for its neuromorphological and cerebral metabolic effects.

The eventual clinical usefulness of NMDA receptor antagonists in cerebral ischaemia may not be determined by their neuroprotective efficacy but by their adverse CNS side-effects. It has been argued that the enormous potential benefits of successful drug treatment with dizocilpine in stroke victims may outweigh the risk to small numbers of CNS neurones (McCulloch & Iversen, 1991). The present studies, however, suggest that NMDA-mediated neuroprotection can be achieved without increases in CMR_{glc} or changes in cortical neuronal morphology by antagonism at the glycine modulatory site.

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