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# The $\sigma$ receptor ligand JO 1784 (igmesine hydrochloride) is neuroprotective in the gerbil model of global cerebral ischaemia

Michael O'Neill [a,*], Maeve Caldwell [a], Bernadette Earley [a], Mark Canney [a], Ambrose O'Halloran [a], John Kelly [a], B.E. Leonard [a], J.-L. Junien [b]

[a] *Department of Pharmacology, University College Galway, Galway, Ireland*
[b] *Institut de Recherche Jouveinal, 94265 Fresnes, Paris, France*

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**Abstract**

To assess the effects of the novel $\sigma$ receptor ligand JO 1784 ((+)-*N*-cyclopropyl-methyl-*N*-methyl-1,4-diphenyl-1-yl-but-3-en-1-ylamine, hydrochloride or igmesine hydrochloride) on behavioural and histological changes following cerebral ischaemia, the gerbil model of cerebral ischaemia was used. Two experiments were carried out. In the first animals were either sham operated, subjected to 5 min of bilateral carotid occlusion or administered JO 1784 (25, 50, 75 or 100 mg/kg p.o.) 1, 24 and 48 h after 5 min bilateral carotid occlusion and histological evaluation carried our 96 h after surgery. In the second experiment the effects of JO 1784 administered at a dose of 100 mg/kg i.p. 30 min, 6, 24 and 48 h post-surgery on home cage activity and nitric oxide (NO) synthase activity in the cortex, hippocampus, cerebellum and brain stem 4 days after surgery was examined. Extensive neuronal death was observed in the CA1 region of 5 min occluded animals. JO 1784 (50, 75 and 100 mg/kg) provided significant protection against this ischaemia-induced cell death ($P < 0.03$–$0.005$). In the second experiment a large increase in home cage activity was observed for 5 min occluded animals for 12 h after surgery ($P = 0.0018$–$0.02$). A large increase in NO synthase activity was observed in all brain regions for 5 min occluded animals. Post-administration of JO 1784 attenuated the ischaemia-induced hyperactivity and increased NO synthase activities. These results show that the selective $\sigma$ receptor ligand JO 1784 is neuroprotective in the gerbil model of cerebral ischaemia and indicates that $\sigma$ receptor ligands may be useful in preventing ischaemia-induced neurodegeneration.

*Keywords:* Cerebral ischemia; JO 1784 (igmesine hydrochloride); Hippocampus; Neuroprotection; Nitric oxide (NO) synthase; (Gerbil)

## 1. Introduction

Neuropathological studies have shown that ischaemia causes a selective pattern of neurodegeneration in man and animals (Brierley, 1976; Ito et al., 1975; Kirino, 1982). The most vulnerable neurons are found in the hippocampus, striatum and certain layers of the cerebral cortex (Crain et al., 1988). Many animal models have been used to evaluate the neuropathological changes induced by cerebral ischaemia and if these changes can be prevented using neuroprotective agents (Gill et al., 1987; Ginsberg and Busto, 1989; O'Neill et al., 1994a,b). The Mongolian gerbil (*Meriones unguiculatus*) is unique in having an incomplete circle of Willis, which allows transient global ischaemia to be induced by bilateral carotid occlusion (BCO) (Kirino, 1982; Gill et al., 1987). The CA1 subfield of the hippocampus is particularly susceptible to ischaemia and brief periods of ischaemia cause neuronal cell death in this region (Crain et al., 1988).

The exact mechanism of ischaemia-induced cell death remains to be elucidated, however depolarisation causes a large increase in neurotransmitters during ischaemia (Globus et al., 1988; Siesjö, 1992). Glutamate is known to increase during ischaemia, and through an action on *N*-methyl-D-aspartate (NMDA) and $\alpha$-amino-3-hydroxy-5-methyl-4-isoazole propionate (AMPA) receptors allows calcium to enter the cell (Watkins and Olverman, 1987; Choi, 1992: McCulloch, 1992). Glutamate also acts on metabotrophic receptors

* Corresponding author. Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey GU20 6PH, England. Tel. +00 44 1276 853538, fax +00 44 1276 853525.

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and leads to the production of diacylglycerol and inositol tri-phosphate (IP3), which activate enzymes and lead to the release of $Ca^{2+}$ from intracellular stores (Watkins and Olverman, 1987; Choi, 1992). $Ca^{2+}$ can also enter through voltage-gated $Ca^{2+}$ channels (Schurr and Rigor, 1992; Siesjö, 1992). This net result is a $Ca^{2+}$ 'overload' which leads to activation of proteases, nucleases, phospholipases, NO synthase and other degradative enzymes that lead to free radical production and cell death (Siesjö, 1992). Support for this hypothesis has come from experiments showing high densities of excitatory amino acid receptors in the CA1 region of the hippocampus (Cotman et al., 1987), and studies that indicate that many compounds acting at these receptors have beneficial effects against cerebral ischaemia (Gill et al., 1987; Iversen et al., 1988; Grotta et al., 1990; Sheardown et al., 1990; Bullock et al., 1994).

Many investigators have shown that the non-competitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclo-hepten-5,10-imine maleate (MK-801) (Gill et al., 1987, 1988; McCulloch, 1992; Hayward et al., 1993), and competitive NMDA receptor antagonists such as *cis*-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS 19755) (Boast et al., 1988; Grotta et al., 1990; Gotti et al., 1990) and 3-(2-carboxypiperazine-4-yl)-prophenyl-1-phosphonate (D-CPPene) (Boast et al., 1988; Park et al., 1992) are neuroprotective in animal models of global and focal cerebral ischaemia. However, other studies have suggested that MK-801 produces its neuroprotective effects by inducing hypothermia (Buchan and Pulsinelli, 1990; Corbett et al., 1990). While others postulate that the protective actions of MK-801 are mediated by a small transient hypothermia that acts synergistically with the drug to yield neuroprotection (Hayward et al., 1993).

Many recent studies have focused on the neuroprotective actions of AMPA receptor antagonists in animal models of cerebral ischaemia (Sheardown et al., 1990; Judge et al., 1991; Diemer et al., 1992; Le Peillet et al., 1992; Bullock et al., 1994; Gill, 1994). Further studies have reported that AMPA receptor antagonists, but not NMDA receptor antagonists are neuroprotective in animal models of cerebral ischaemia (Nellgård and Wieloch, 1992; Sheardown et al., 1993; Xue et al., 1994).

Other studies have shown that $Ca^{2+}$ channel blockers, free radical scavengers and lipid peroxidation inhibitors have neuroprotective effects in cerebral ischaemia (Hall et al., 1988; Elben et al., 1992). The availability of several synthetic $\omega$-conopeptides such as synthetic MVIIA (SNX-111) has provided the opportunity to evaluate the therapeutic potential of selectively blocking N-type calcium channels in a variety of pathological conditions including cerebral ischaemia. SNX-111 has recently been shown to have neuroprotective effects in rat models of focal ischaemia (Smith and Siesjö, 1992; Zhao et al., 1994). Nitric oxide synthase inhibitors have also been examined as possible neuroprotective agents (Nowicki et al., 1991; Buisson et al., 1993; Caldwell et al., 1994). $N^G$-Nitro-L-arginine has given neuroprotective effects in global (Caldwell et al., 1994) and focal ischaemia (Nowicki et al., 1991). Protective effects with $N^G$-nitro-L-arginine methyl ester have also been reported (Buisson et al., 1993). However other investigators report that NO synthase inhibitors enhance damage in global (Weissman et al., 1992) and focal ischaemia (Yamamoto et al., 1992).

Recently the role of $\sigma$ ligands as neuroprotective agents has been investigated (Contreras et al., 1992; Clissold et al., 1993; O'Neill et al., 1994a,b). These ligands bind to $\sigma$ receptors, which are non-opiate binding sites with a high affinity for (+)-opiates and certain antipsychotic drugs (Martin et al., 1976; Junien and Leonard, 1989; Walker et al., 1990, 1992). It has been shown that (+)-SKF 10,047 protects against ischaemia-induced neuronal damage in gerbils (Lysko et al., 1992). $\alpha$-(4-Fluorophenyl)-4-(5-fluoro-2-pyramidinyl)-1-piperazine butanol (BMY 14802) administered (10, 30 or 50 mg/kg i.p.) 30 min prior to 5 min BCO has also provided neuroprotective effects in the gerbil (Contreras et al., 1992). JO 1784 (igmesine hydrochloride or (+)-*N*-cyclopropyl-methyl-*N*-methyl-1,4-diphenyl-1-yl-but-3-en-1-ylamine, hydrochloride) is a selective in vitro and in vivo $\sigma$ receptor ligand (Roman et al., 1990; Quirion et al., 1992). It has been shown that JO 1784 has anti-amnesic effects in rats (Earley et al., 1991) and preliminary studies have shown that JO 1784 has neuroprotective actions (Earley et al., 1993). Results from our laboratory also indicate that JO 1784 can attenuated trimethyltin-induced memory deficits and receptor dysfunction in rats (O'Connell et al., submitted).

To evaluate the effects of JO 1784 in the gerbil model of cerebral ischaemia two experiments were carried out: (1) to examine the effects of JO 1784 administered at 25, 50, 75 and 100 mg/kg p.o. 1, 24 and 48 h after bilateral carotid occlusion on histological parameters 96 h affter surgery and (2) to examine the effects of JO 1784 (100 mg/kg i.p.) administered 30 min, 6, 24 and 48 h after surgery on home cage activity for 2 days after surgery and NO synthase activity in 4 brain regions measured 4 days after surgery.

## 2. Materials and methods

### 2.1. Animals and surgery

Male Mongolian gerbils (Bantin and Kingman, Hull, UK) at least 3 months old and weighing in excess of 60

g were used. The animals were maintained in standard lighting conditions and food and water were available ad libitum. The animals were anaesthetised with a 5% halothane-oxygen mixture and maintained using 2% halothane delivered with oxygen at 1l/min via a face mask throughtout the operation. Through a midline cervical incision, both common carotid arteries were exposed and freed from surrounding connective tissue. In animals to be rendered ischaemic, a short length of salastic tubing was inserted under each artery, and the occlusion itself produced by twisting the tubing 3–4 times. This effectively occluded the artery. At the end of the occlusion period (5 min) blood flow was re-established by uncoiling the tubing and removing it. In sham operated animals the arteries were exposed but not occluded. The wound was then sutured and the animals allowed to recover. The temperature was maintained at 37°C using a CMA/150 temperature controller/heating pad (Carnigie Medicin, Sweden) and brain temperatures were maintained using a heating lamp. In the first experiment JO 1784 was administered at 25, 50, 75 or 100 mg/kg p.o. 1, 24 and 48 h after occlusion. In the second experiment JO 1784 was administered 100 mg/kg i.p. 30 min, 6, 24 and 48 h post-occlusion.

### 2.2. Home cage activity

Home cage activity was monitored for 2 days before (baseline) and 2 days after surgery. The animals were singly housed 2 days prior to placement in the apparatus which consisted of two monitors each having the capacity to measure 24 animals. The animals were placed in indivual compartments and an infrared sensor positioned over each cage measured the movement in that cage [(c) A. O'Halloran, Pharmacology Department, U.C.G., Ireland (1992)]. Measurements were taken every 15 min for baseline monitoring and every 1 min after surgery and statistical analyses carried out using lotus 123 and minitab (V 8.2).

### 2.3. Nitric oxide synthase activities

*Sample preparation*

After completion of behavioural studies, the animals were killed 96 h post surgery by decapitation and the brain were removed and dissected. The cerebellum, brain stem, striatum, hippocampus and cerebral cortex were rapidly dissected on ice, weighed and placed in 1 ml of buffer containing Tris-HCl (50 mM, pH 7.4), EDTA (1 mM), antipain (10 mg/l), leupeptin (10 mg/l), Soybean trypsin inhibitor (10 mg/l), pepstatin (10 mg/l), chymostatin (10 mg/l) and phenylmethylsulphonyl fluoride (100 mg/l) and sonicated. Homogenates were centrifuged at 21 000 × g for 30 min. Supernatants were removed and stored at −20°C until use.

*Assay of nitric oxide synthase*

NO synthase activity was measured by monitoring the conversion of [$^3$H]L-arginine to [$^3$H]L-citrulline (Bredt and Synder; 1989). 50 μl of enzyme extract, 25 μl of 40 mM L-arginine and 25 μl of 100 nm [$^3$H]-L-arginine was added to buffer containing 50 mM Hepes (pH 7.4), 1 mM NADPH, 1 mM EDTA, 1.25 mM $CaCl_2$, 1 mM dithiothretiol and 10 μg of calmodulin per ml. After incubation at 22°C for 5 min the assay was terminated with the addition of 2 ml of 20 mM Hepes (pH 5.5) which was then applied to 1 ml columns of Dowex AG50X-8 ($Na^+$ form), and eluted with 2 ml of water. [$^3$H]L-citrulline was quantified by liquid scintillation spectroscopy of 4 ml flow through. Blanks used in the studies were incubations containing no enzyme extract. Protein was determined by the method of Bradford (1976).

### 2.4. Histology

The animals were sacrificed 4 days after surgery and the brains were removed, processed and embedded in parafin wax. 5 μm coronal sections were taken 1.5–1.7 mm caudal to the bregma in the anterior hippocampus using a microtome (Reichert-Jung, Biocut 2035). The slices were stained with cresyl violet and the neuronal density in the CA1 subfield of the hippocampus was

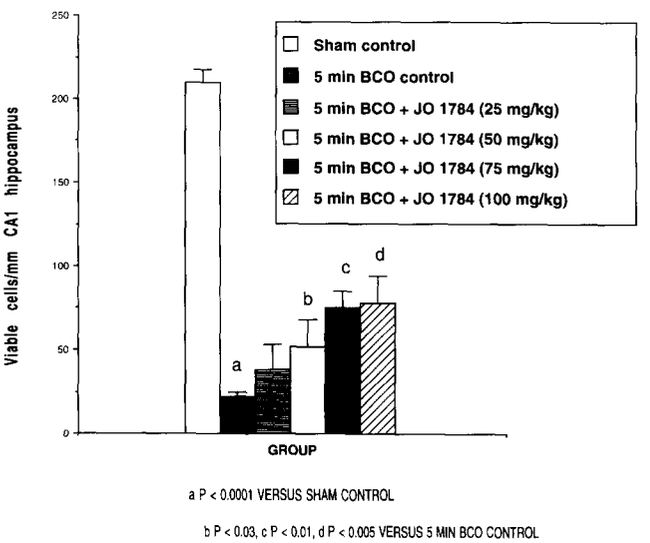


Fig. 1. The effects of JO 1784 administered 1 h before 5 min bilateral carotid occlusion on the neuronal cell density in the CA1 region of the hippocampus 4 days after surgery. Results are expressed as means ± S.E.M. viable cells/mm CA1 hippocampal region ($n = 10$ animals per group). 5 min BCO caused a severe loss in neuronal cells in the CA1 region ($P < 0.0001$). The 25 mg/kg dose of JO 1784 provided some neuroprotection, while the 50, 75 and 100 mg/kg doses of JO 1784 gave significant protection against the ischaemia-induced cell death in the hippocampus ($P < 0.03$, $P < 0.01$ and $P < 0.005$, respectively). Student's $t$-test with Bonferroni correction factor.

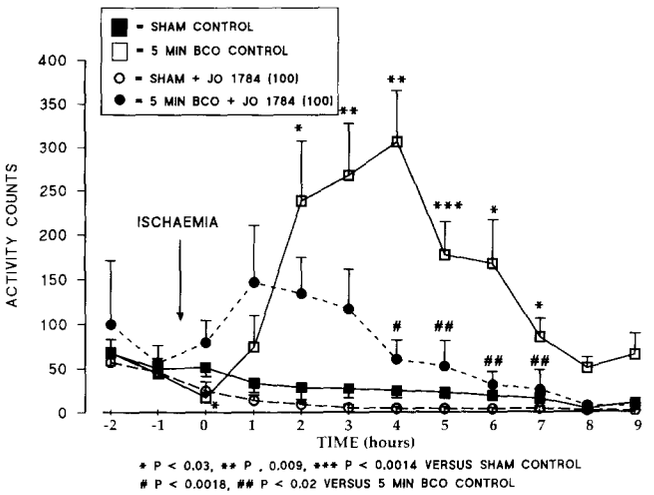


Fig. 2. The effects of JO 1784 on home cage activity counts in the gerbil. A large ischaemia-induced increase in hyperactivity was observed for 5 min BCO animals ($P < 0.0014$–$0.03$). JO 1784 (100 mg/kg i.p.) attenuated this ischaemia-induced hyperactivity ($P < 0.0018$–$0.02$). Mann-Whitney U-test.

measured using a microscope with grid lines (0.05 mm × 0.05 mm).

### 2.5. Statistics

Statistical analysis was carried out using Lotus 123 and Minitab routines. Behavioural data was analysed using Kruskal Wallis ANOVA (Kruskal and Wallis, 1952) followed by Mann Whitney U-test (Mann and Whitney, 1947) where appropriate. Two-tailed tests of significance were used in evaluating all comparisons. Statistical analysis of biochemical and histological data was carried out using ANOVA followed by Student's $t$-test with Bonferroni corrections using $P < 0.05$ as the level of significance.

## 3. Results

### 3.1. Histological results

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 CA1 pyramidal neurons were found to be degenerated in the 5 min occluded animals. The neuronal death involved nearly all the pyramidal neurons and this neurodegeneration was not evident in any other forebrain region. The pyramidal cell density was counted at three different points along the CA1 region of the hippocampus and the results expressed as mean ± S.E.M. neuronal density per 1 mm CA1 (Fig. 1). The results indicated that there was severe loss of neurons in the CA1 region of the hippocampus of 5 min occluded animals. The 25

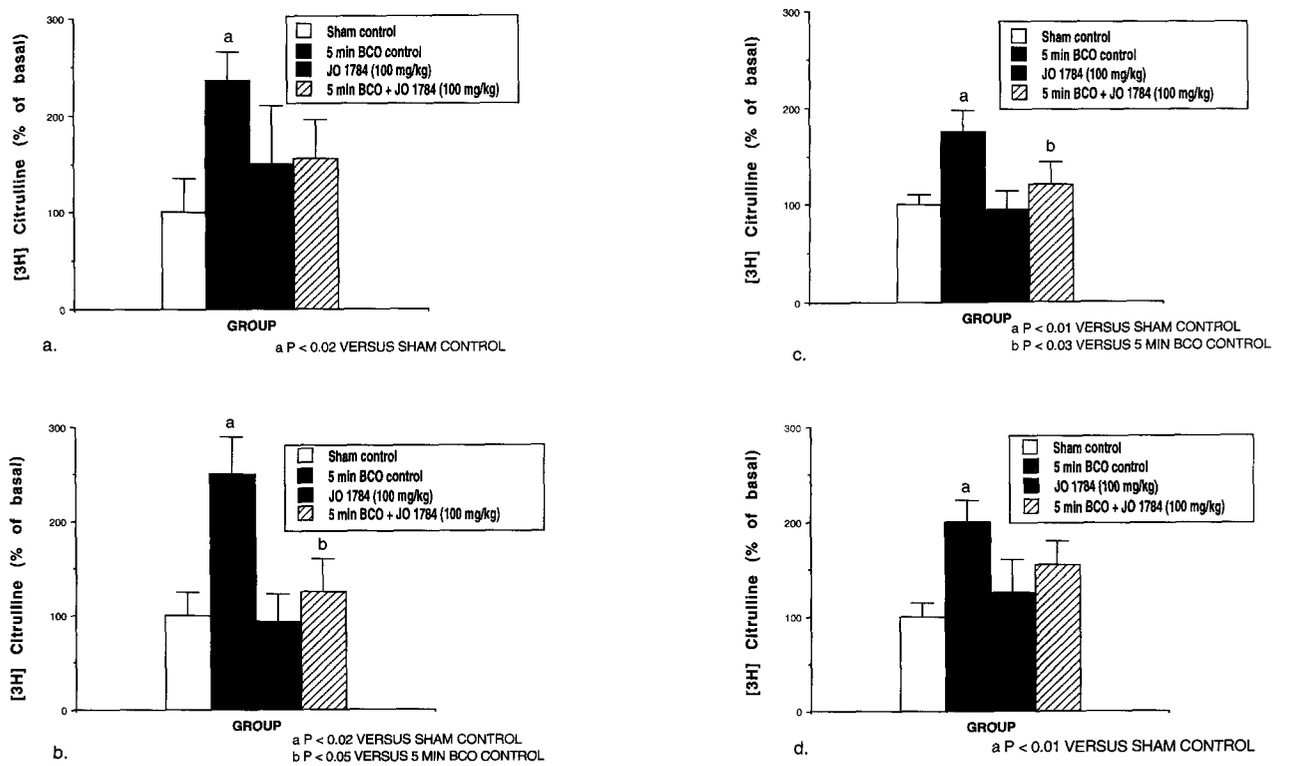


Fig. 3. The effects of JO 1784 on ischaemia-induced changes in NO synthase activities in the cerebellum (a), brain stem (b), hippocampus (c) and cortex (d) 4 days after occlusion in the gerbil. A large increase in NO synthase activities was observed in all regions after 5 min BCO. JO 1784 attenuated the ischaemia-induced increases in NO synthase activities and this was significant in the brain stem (Fig. 3b) and hippocampus (Fig. 3c). $n = 8$ animals per group.

mg/kg dose of JO 1784 gave some protection against this ischaemia-induced cell death, while the 50, 75 and 100 mg/kg doses provided significant neuroprotection (Fig. 1).

### 3.2. Home cage activity

No changes in baseline home cage activity counts were observed between groups for 2 days prior to surgery. There was a large increase in home cage activity counts for 5 min BCO animals 2 h after occlusion (Fig. 2). The hyperactivity was maximal for 2–7 h post-occlusion. Post-administration of JO 1784 (100 mg/kg i.p.) significantly attenuated this ischaemia-induced hyperactivity (Fig. 2).

### 3.3. NO synthase activities

A large increase in NO synthase activity was observed in the hippocampus, cerebellum, brain stem, striatum and cortex of 5 min bilateral carotid occluded animals compared to sham controls (Fig. 3a–d). This increase was attenuated in all brain regions by JO 1784 and this attenuation was statistically significant in the hippocampus and brain stem (Fig. 3a–d).

## 4. Discussion

In the present experiment a large increase in home cage activity was observed 2 h after occluion of the carotid arteries. This hyperactivity was present for 7 h after occluion and as the animals were allowed habituate in a singly housed home environment prior to surgery the hyperactivity is probably due to the ischaemia. We have previously shown increased locomotor activity 1, 2 and 3 days after 5 min of cerebral ischaemia in the gerbil (O'Neill et al., 1993, 1995; Caldwell et al., 1994). Other investigators have shown increased locomotor activity after ischaemia in the gerbil (Chandler et al., 1985; Gerhardt and Boast, 1988; Mileson and Schwartz, 1991). Katoh and coworkers (Katoh et al., 1992) found that the hyperactivity caused by 5 min of occlusion returned to control values 5 days post-surgery. However, other investigators have reported hyperactivity 24 days after 5 min bilateral carotid occlusion (Mileson and Schwartz, 1991). In the present study we found that the ischaemia-induced increases in home cage activity developed immediately after the animals recovered from the anaesthetic. The hyperactivity was only observed in the first 12 h after surgery and suggests that as the animals were singly housed in their home environment they adaped to the insult quickly (Fig. 2). Previous studies support this finding and it has been shown that 5 days exposure to the open field can block the development of increased locomotor activity (Wang and Corbett, 1990). Other recent studies have indicated that if ischaemic animals are tested every day for 14 days in the locomotor activity apparatus no hyperactivity is observed on days 13 or 14 (Babcock et al., 1993). However, if similarly treated animals are exposed to the test only on days 13 and 14 a large hyperactivity is observed in ischaemic animals.

Although the exact cause of the increase in locomotor activity observed in the above studies is not clear, it may be that the large release of neurotransmitters (particularly dopamine and glutamate) together with the severe damage to the hippocampal region may contribute to this hyperactivity. In the present experiments JO 1784 attenuated the ischaemia-induced increase in home cage activity. We have previously shown that compounds that attenuate ischaemia-induced increases in locomotor activity also provided neuroprotection (Caldwell et al., 1994; O' Neill et al., 1995). It has also been shown that neuroprotective agent, NBQX, attenuates ischaemia-induced hyperactivity and prevents neuronal cell death in the gerbil (Judge et al., 1991).

In the present study severe damage to the CA1 neurons was observed in occluded animals, while other regions remained intact. This is in accordance with several studies which have shown selective neurodegeneration after transient forebrain ischaemia in the gerbil (Kirino, 1982; Kirino and Sano, 1984; Crain et al., 1988). Many studies have tried to prevent this cell death using NMDA receptor antagonists, AMPA receptor antagonists, free radical scavengers, lipid peroxidation inhibitors and calcium antagonists (Gill et al., 1987; Hall et al., 1988; Elben et al., 1992; Bullock et al., 1994). Initial studies focused on NMDA receptor antagonists as neuroprotective agents (Gill et al., 1987; McCulloch, 1992). However the discovery that MK-801 caused hypothermia led to the suggestion that MK-801 was providing neuroprotection by producing hypothermia (Buchan and Pulsinelli, 1990; Corbett et al., 1990).

In 1990 Sheardown and co-workers found that 2,3-dihydroxy-6-nitro-7-sulfamoyl-bennzo(*F*)quinoxaline (NBQX), a competitive AMPA/kainate receptor antagonist was neuroprotective in the gerbil. Further studies by the same group found that NBQX (30 mg/kg i.p.) administered at 60, 70 and 85 min after 5 min bilateral carotid occlusion protected from both hippocampal damage and post-ischaemic hyperactivity (Judge et al., 1991). It was later shown that delayed treatment with NBQX protected against ischaemic damage in gerbils (Sheardown et al., 1993) and rats (Li and Buchan, 1993). The non-competitive AMPA/kainate receptor antagonist 1-(aminophenyl)-4-methyl-7,8-methylenediooxy-5*H*-2,3-benzodiazepine (GYKI 52466) has also been shown to have neuroprotective effects in animal models of global (Le Peillet et al.,

1992) and focal (Smith and Meldrum, 1992; Xue et al., 1994) cerebral ischaemia.

Recently, it has been shown that (3*SR*,4a*RS*,6*RS*, 8a*RS*)-6-[2-(1*H*-tetrazol-5-yl)-ethyl]-1,2,3,4a,5,6,7,8a-decahydroisoquinoline-3-carboxylic acid (LY-215490), a novel selective competitive antagonist of AMPA/kainate receptors has been shown to be protective against focal ischaemia in the rat (Gill and Lodge, 1995). It has also been shown that the active isomer of LY 215490, which is LY 293558 is neuroprotective in a cat model of cerebral ischaemia (Bullock et al, 1994).

The role of $\sigma$ ligands as neuroprotective agents has also been investigated (Taylor et al, 1992; Contreras et al., 1992; O' Neill et al., 1994a,b). In the present studies JO 1784 protected against the ischaemia-induced neuronal damage and attenuated the ischaemia-induced increased NO synthase activities in several brain regions. We have previously shown that the NO synthase inhibitor $N^G$-nitro-L-arginine prevents the increased NO synthase activity caused by ischaemia in gerbil brain (Caldwell et al., 1994). Ischaemia is known to release glutamate, which acts on the NMDA and AMPA receptors to initiate a cascade of calcium-mediated toxicity, lipid peroxidation and free radical production (Siesjö, 1992). It has recently been shown that activation of the NMDA receptor activates NO synthase, which leads to excess production of $NO^{\cdot}$ (Moncada et al., 1991; Garthwaite et al., 1989). High concentrations of $NO^{\cdot}$ are toxic and interact with $O_2^{\cdot}$ to produce the highly toxic peroxynitrite anion ($ONOO^-$) (Beckman et al., 1990). Nitric oxide synthase inhibitors have also been examined as possible neuroprotective agents (Nowicki et al., 1991; Caldwell et al., 1994; Dawson et al., 1992; Yoshida et al., 1994). $N^G$-Nitro-L-arginine has given neuroprotective effects in global (Caldwell et al., 1994) and focal ischaemia (Nowicki et al., 1991). Protective effects with $N^G$-nitro-L-arginine methyl ester have also been reported (Buisson et al., 1993). However other investigators report that NO synthase inhibitors enhance damage in global (Weissman et al., 1992) and focal ischaemia (Dawson et al., 1992; Yamamoto et al., 1992). NO synthase inhibitors have the ability to increase blood pressure due to their blockade of NO in the vascular endothelia and this could influence outcome after ischaemia (Moncada et al., 1991; Caldwell et al., 1994). It has been suggested that lower doses of NO synthase inhibitors may inhibit the cerebral NO synthase with little effect on the endothelial enzyme and this may explain why low doses have given protective effects and higher doses have enhanced the damage (Buisson et al., 1993). From our studies it is clear that the dose and time of administration are crucial factors and in studies that have used high doses prior to occlusion an increase in damage is to be expected (Weissmann et al., 1992). The recent findings that 7-nitroindazole (7-NI) which inhibits neuronal NO synthase without any effects on blood pressure is neuroprotective in focal ischaemia may lead to compounds that may be useful anti-ischaemic agents (Yoshida et al., 1994). The ability of JO 1784 to reduce the ischaemia-induced increases in NO synthase suggests that this compound may be providing neuroprotective action by interacting with $\sigma$ receptors and this may modulate the NMDA receptor complex.

Support for this concept comes from studies that indicate that there are dense binding sites for JO 1784 in the pyramidal and granule layers of the hippocampus (Roman et al., 1990; Junien et al., 1991). It is well known that there is a high density of excitatory amino acid receptors in these regions (Cotman et al., 1987). Other work has shown that ifenprodil (a compound with affinity for $\sigma$ receptors) reduces the infarct volume following focal ischaemia in the mouse (Gotti et al., 1988, 1990). Pontecorvo and co-workers (1991) reported that ifenprodil and several other $\sigma$ receptor ligands increased survival time in a hypoxic environment. Although it is known that ifenprodil is a non-competitive NMDA receptor antagonist acting at the polyamine site they suggested that other $\sigma$ receptor ligands may have similar functional and cerbroprotective ifenprodil-like effects (Pontecorvo et al., 1991). It has also been shown that eliprodil (SL 82.0715), another NMDA receptor antagonist which acts at the polyamine site has affinty for $\sigma$ receptors (Gotti et al., 1988; Poignet et al., 1992). Eliprodil has also given protective effect in animal models of cerebral ischaemia and traumatic brain injury (Poignet et al., 1992; Toulmond et al., 1993).

We have shown that BMY 14802 and dextromethorphan protect against ischaemia-induced cell death in the CA1 region of the hippocampus (O'Neill et al., 1994a, b). In the present studies we found protective effects with JO 1784 which has been shown to be a selective $\sigma$ receptor ligand with an IC50 of $39 \pm 8$ nM and virtually no affinity for phencyclidine sites (Roman et al., 1990; Quirion et al., 1992). It has been shown that $\sigma$ receptor ligands such as 1,3-di(2-tolyl) guanidine and JO 1784 can selectively enhance NMDA-induced activation of CA3 hippocampal pyramidal cells (Monnet et al., 1990). Earley et al. (1991) have reported that JO 1784 significantly reversed scopolamine-induced amnesia in rats.

Recent studies have shown that (+)-*N*-allylnormetazocine (NANM, (+)-SKF 10,047) protects against ischaemia-induced damage in the gerbil (Lysko et al., 1992). Other evidence has shown that BMY 14802, ifenprodil and dextromethorphan block NMDA-induced seizures (Kaiser et al., 1991). Clissold and collaborators (1993) have reported that 6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methylflavone hydrochloride (NPC 16377) a potent a selective $\sigma$ receptor ligand is

neuroprotective in the gerbil. As NPC 16377 did not antagonise NMDA-induced siezures in mice and did not directly antagonise NMDA-evoked currents in the *Xenopus* oocyte expression system they suggested that $\sigma$ receptor ligands alone may have intrinsic neuroprotective actions (Clissold et al., 1993).

Some studies have reported a lack of neuroprotection with $\sigma$ receptor ligands, but as pointed out by the authors of theses studies the compounds tested may have being acting as agonists (Poignet et al., 1992). The most recent results indicate that $\sigma$ receptor ligands indirectly modulate the NMDA receptor-ion channel complex through the $\sigma^1$ site in vivo (Yamamoto et al., 1995). It has also been shown that $\sigma$ receptor antagonists produce a concentration-dependent attenuation of the hypoxia/hypoglycemia-induced decrease in CA1 presynaptic fiber spikes in rat hippocampal slices (Shibata et al., 1995). The same group have also shown that $\sigma$ receptor agonists such as DTG potentiate the decrease in fiber spikes (Shibata et al., 1995).

In conclusion, in the present studies the selective $\sigma$ receptor ligand JO 1784 protected against ischaemia-induced behavioural, biochemical and histological damage in the gerbil. Preliminary results from our laboratory indicate that JO 1784 reduces the infarct volume and attenuates ischaemia-induced increases in [$^3$H] PK 11195 binding in the cortex following permanent focal ischaemia in the mouse (Earley et al., unpublished results). Therefore the $\sigma$ receptor may have important effects in cerebral ischaemia and although the their exact mechanism(s) of action remains uncertain, $\sigma$ receptor ligands may play a role as anti-ischaemic agents.

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