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Short communication

N^G-Nitro-L-arginine methyl ester protects against lipid peroxidation in the gerbil following cerebral ischaemia

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Abstract

The aim of this study was to assess the role of nitric oxide (NO) in lipid peroxidation following 5 min of bilateral carotid occlusion in the Mongolian gerbil. The study consisted of 4 experimental groups ($n = 10$). Animals were either sham operated, subjected to bilateral carotid occlusion or administered the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg i.p.) 30 min, 6, 24 and 48 h following sham operation or 5 min bilateral carotid occlusion. Animals were killed 96 h post surgery and changes in the concentrations of malonaldehyde and 4 hydroxyalkenals (the main decomposition products of peroxides derived from polyunsaturated fatty acids and related esters) were measured in the hippocampus and cortex using the LPO-586 colorimetric method. The results showed a significant increase in the concentrations of both decomposition products following 5 min of bilateral carotid occlusion. L-NAME administered to sham operated controls had no effect, but in those animals subjected to 5 min of bilateral carotid occlusion L-NAME significantly decreased the levels of both decomposition products. These results suggest that inhibition of NO synthase activity decreases lipid peroxidation in the gerbil model of cerebral ischaemia.

Keywords: Cerebral ischemia; Hippocampus; Lipid peroxidation; N^G-Nitro-L-arginine methyl ester; (Gerbil)

1. Introduction

In 1966, the Mongolian gerbil (*Meriones unquilatus*) was introduced as a model of cerebral ischaemia (Levine and Payan, 1966). The gerbil is unique in having an incomplete circulus of Willis which allows transient global ischaemia to be induced by bilateral carotid artery occlusion (Levine and Payan, 1966). Occlusion of the common carotid arteries for 5 min causes a selective pattern of neurodegeneration with the CA1 pyramidal region of the hippocampus being particularly susceptible (Levine and Payan, 1966). This model is often used for testing putative cerebroprotective compounds.

There is now extensive experimental evidence to support the early occurrence and importance of oxygen radical formation and cell membrane lipid peroxidation in central nervous system injury (Braughler and Hall, 1989; Hall and Braugher, 1989). Lipid peroxidation is an event which could be expected to induce marked changes in membrane structure and function. Free radicals are a species in which one or more of the outer electron orbitals contains only a single electron rather than the usual pair and are capable of inducing damage to lipids, proteins and nucleic acids (Floyd, 1990; Kontos, 1989). The role of nitric oxide (NO), which is formed as a consequence of glutamate receptor activation during ischaemia, has been extensively investigated. High concentrations of NO are toxic and increased concentrations interact with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) (Beckman et al., 1990). These observations formed the basis of the present study in which L-NAME, an inhibitor of nitric oxide

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synthase which is responsible for the production of NO from L-arginine was investigated to see if it would attenuate the lipid peroxidation that resulted from cerebral ischaemia in the gerbil.

2. Materials and methods

2.1. Animals and surgery

Male Mongolian gerbils (obtained from 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 (standard laboratory diet composition) and water were freely available. To evaluate the neuroprotective effect of L-NAME, four groups ($n = 10$) were used. The animals were either sham-operated, subjected to 5 min BCO or administered L-NAME (10 mg/kg i.p.) 30 min 6, 24 and 48 h following either sham operation or 5 min of BCO. Animals were killed 96 h post surgery and hippocampus and cortex were removed to determine the concentrations of malonaldehyde and 4-hydroxyalkenals.

The animals were anaesthetised with 5% halothane delivered with oxygen and maintained using 2% halothane delivered with oxygen at 1 l/min via a face mask throughout the operation. Through a ventral 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 Silastic 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 of 5 min blood flow was re-established by uncoiling the tubing and removing it. The wound was then sutured and animals were allowed to recover. The body temperature was maintained at 37°C using a CMA/150 temperature controlled heating pad.

2.2. Lipid peroxidation assay

Malonaldehyde and 4-hydroxyalkenals in a single aqueous sample were measured by the LPO-586 method (Bioxytech, France). The LPO-586 method is based on a chromogenic reagent at the concentration of 10.3 mM, in acetonitrile, which reacts with malonaldehyde and 4-hydroxyalkenals at 45°C. Condensation of one molecule of either malonaldehyde or 4-hydroxyalkenals with 2 molecules of this chromophoric reagent yields a stable chromophore with maximal absorbance at the 586 nm wavelength.

2.3. Statistics

Statistical analyses were performed using Lotus 123 and minitab routines. Student's t-test (two-tailed) was used, taking $P < 0.05$ as the level of significance.

3. Results

The results showed a significant increase in the concentrations of both decomposition products malon-

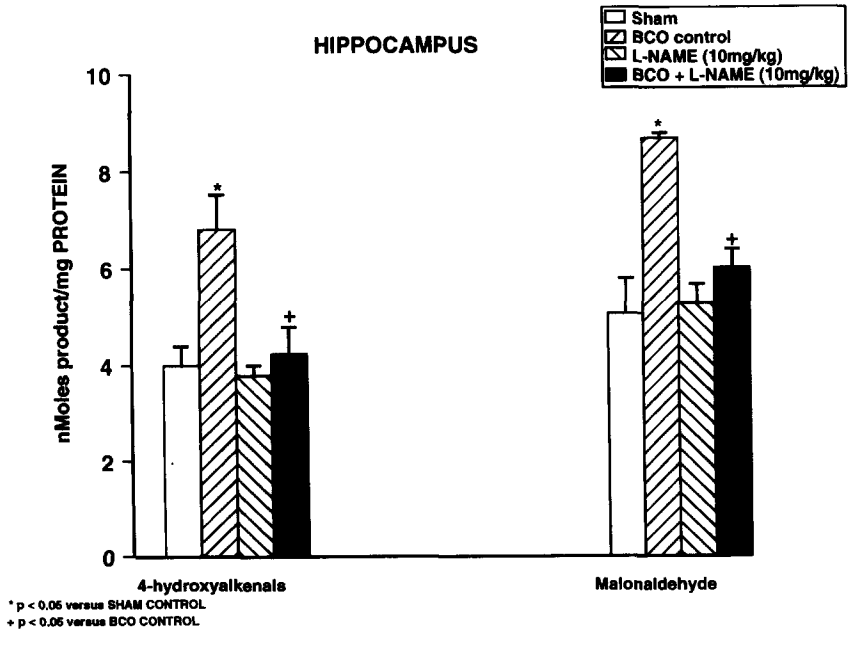

Fig. 1. Effect of 5 min bilateral carotid occlusion and administration of L-NAME (10 mg/kg) on levels of malonaldehyde and 4-hydroxyalkenals in the hippocampus.

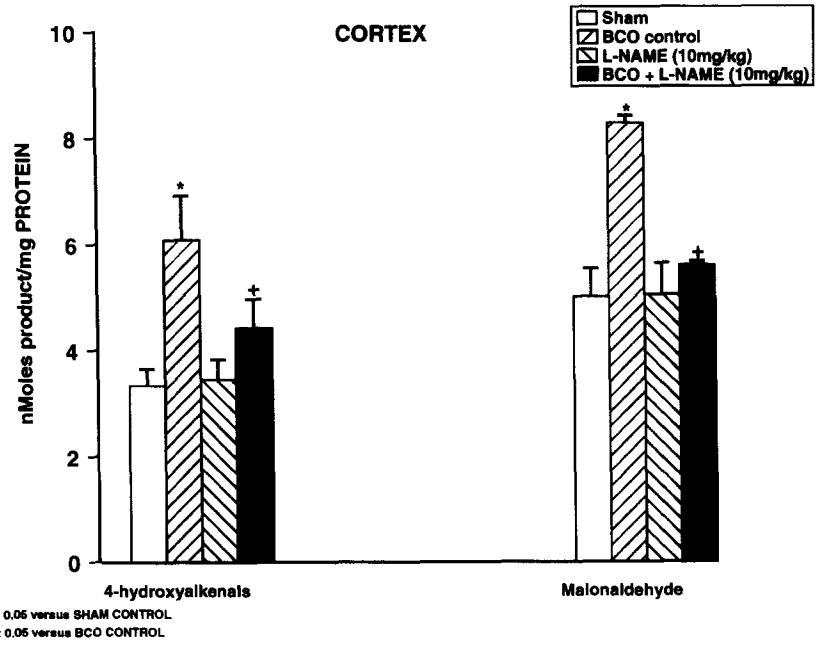

Fig. 2. Effect of 5 min bilateral carotid occlusion and administration of L-NAME (10 mg/kg) on levels of malonaldehyde and 4-hydroxyalkenals in the cortex.

aldehyde and 4-hydroxyalkenals following 5 min of bilateral carotid occlusion in the hippocampus (Fig. 1) and the cortex (Fig. 2). L-NAME administered to sham operated controls had no effects while L-NAME administered to animals subjected to 5 min of bilateral carotid occlusion significantly decreased the levels of the decomposition products in both brain regions measured.

4. Discussion

It can be hypothesized that stimulation of the *N*-methyl-D-aspartate (NMDA) receptor by an ischaemic insult may raise levels of cytosolic Ca^{2+} which could lead to the activation of NO synthase and overproduction of NO (Garthwaite, 1991). Because NO contains an unpaired electron it rapidly reacts with superoxide anions (O_2^-) to produce peroxynitrite ($ONOO^-$). These anions oxidise sulphydryl groups and peroxidise membrane lipids (Blough and Zafiriou, 1985; Radi et al., 1991) and decompose to yield the hydroxyl radical ($OH^\cdot$) and nitrogen dioxide (Beckman et al., 1990).

The role of NO in neuronal toxicity has recently been discovered (Dawson et al., 1991; Moncada et al., 1990), leading to the suggestion that NO synthase inhibitors have neuroprotective activity. The dose of the NO synthase inhibitor, L-NAME, used in this study is based on findings by other groups. Systemic administration of a low dose of N^G-nitro-L-arginine (L-NNA) decreased dramatically the size of cortical infarction induced after mouse middle cerebral artery occlusion (Nowicki et al., 1991). Similar findings were reported in a rat model of cerebral ischaemia where administration of a low dose of L-NAME and L-NNA was found to be neuroprotective (Buisson et al., 1992, 1993); L-NNA was also found to be neuroprotective in the gerbil model of global cerebral ischaemia (Caldwell et al., 1994).

In the present study, the significant attenuation of lipid peroxidation by the NO synthase inhibitor L-NAME would further support the role of free radical damage in the pathology of cerebral ischaemia.

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