The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia

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Recent data showed that glutamate toxicity in primary cortical cultures is mediated by nitric oxide. In order to investigate the effect of inhibition of NO synthase on focal cerebral ischaemia in rats, we studied the histological consequences of a middle cerebral artery (MCA) occlusion after post-operative treatment with N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase. We found a significant reduction of cortical (–43%) and striatal (–25%) necrotic volumes induced by MCA occlusion, indicating that NO synthesis plays an important role in the neurotoxic cascade leading to neuronal damage after focal cerebral ischaemia in rats.

Keywords: Cerebral focal ischaemia in rats; nitric oxide; NO synthase inhibitor

Introduction Nitric oxide (NO) is an ubiquitous messenger molecule which has been shown to be produced enzymatically in the central nervous system in response to activation of N-methyl-D-aspartate (NMDA) receptors. This activation results in a raised influx of Ca²⁺ ions into post-synaptic structures, which in turn activates NO-synthase under physiological conditions (Snyder, 1991).

As the overstimulation of NMDA receptors by excitatory amino acids may initiate several phenomena which lead to neuronal death during cerebral ischaemia (Choi, 1991), and as the blockade of NO synthesis reduces the glutamate-induced neurotoxicity in cultured cortical neurones (Dawson *et al.*, 1991), it might be hypothesized that NO would mediate, at least in part, the ischaemia-induced neuronal damage. In order to investigate the possibility that NO participates in the neuronal damage induced by a focal cerebral ischaemia, we studied the effect of the systemic administration of a competitive inhibitor of NO-synthase, N^G-nitro-L-arginine methyl ester (L-NAME) (Rees *et al.*, 1990), on the histological consequences of permanent occlusion of the MCA in rats.

Methods Male Sprague Dawley rats (280–300 g) were anaesthetized with chloral hydrate (300 mg kg^{–1}, i.p.) and the left MCA was cauterized at its proximal site. Rectal and temporal muscle temperatures were maintained at 36–37°C during the experiment. L-NAME was administered (1 or 3 mg kg^{–1}, i.p.), 5 min, 3 h, 6 h, 24 h and 36 h after ischaemia. The rats were killed 2 days later. Their brains were removed and frozen in isopentane (–40°C). Cryostat coronal brain sections were stained with cresyl violet and analysed with an image analyser (IMSTAR, PARIS). Twelve coronal slices were digitized for each animal. The areas of ischaemic damage were measured by outlining the necrotic cortical and striatal regions on the digitized image and measuring the enclosed area. By using values for the distances between respective coronal sections, a linear integration was used to calculate the cortical and striatal necrotic volumes.

In other groups of rats subjected to the same experimental condition of anaesthesia, mean arterial blood pressure (MABP) was continuously recorded with a chronically implanted femoral catheter.

Results MCA occlusion in rats induced a highly reproducible infarction that affected both the cortex and the striatum. The total volume (mean ± s.e.mean) of the infarction in the vehicle-treated rats was 194 ± 14 mm³ (*n* = 9). The low dose (1 mg kg^{–1}, i.p.) of L-NAME failed to induce any significant change in the infarcted volume in ischaemic rats. Treatment with 3 mg kg^{–1} of L-NAME significantly reduced the total infarct volume by 35% (125 ± 18 mm³, *n* = 9, *P* < 0.05), the cortical infarct volume by 43% (84 ± 16 mm³ versus 139 ± 15 mm³, *P* < 0.05) and the striatal infarct volume by 25% (41 ± 5 mm³ versus 55 ± 4 mm³, *P* < 0.05) (Figure 1).

In chloral anaesthetized rats, treatment with L-NAME at 1 mg kg^{–1} did not induce any significant modification of MABP. Under the same conditions, a hypertension (21 ± 6 mmHg) appeared 30 min after treatment with L-NAME 3 mg kg^{–1} (Figure 2). The repeated treatment at 3 h,

Figure 1 Effect of middle cerebral artery occlusion on striatal, cortical and total cerebral necrotic volumes developed by control rats (solid columns), and by rats treated with N^G-nitro-L-arginine methyl ester (L-NAME) 1 mg kg^{–1}, i.p. (hatched columns) and 3 mg kg^{–1}, i.p. (open columns) administered 5 min, 3 h, 6 h, 24 h and 36 h after induction of cerebral focal ischaemia. Each column is the mean of 8–9 experiments with s.e.mean shown by vertical bars. Statistical significance was assessed by ANOVA followed by a Dunnett test. **P* < 0.05; NS, not significant.

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Figure 2 Mean arterial blood pressure of chloral anaesthetized rats, treated with N^G -nitro-L-arginine methyl ester (L-NAME) 1 mg kg^{-1} , i.p. (●), 3 mg kg^{-1} , i.p. (□) or vehicle-treated rats (○). Each point is the mean of 5 experiments with s.e.mean shown by vertical bars. Statistical significance was assessed by ANOVA followed by a Dunnett test. * $P < 0.05$.

6 h, 24 h and 36 h on rats that were awake did not induce any modification of MABP.

Discussion The infarcted volume obtained in our control Sprague Dawley rats is consistent with the recent data of Mackay *et al.* (1991) obtained after MCA occlusion in the same strain. The present study represents the first demonstration that blockade of NO synthesis markedly reduces the cerebral infarct volume induced by a cauterisation of the

MCA in rats. These results are in accordance with those of Nowicki *et al.* (1991), showing that in mice, a post treatment with N^G -nitro-L-arginine protects the cortex from ischaemic insult induced by irreversible focal ischaemia. The hypertension observed in anaesthetized rats after the first treatment with L-NAME (3 mg kg^{-1}) is a consequence of the inhibition of endothelial NO-synthase (Rees *et al.*, 1990). However, as Jacewicz *et al.* (1990) have shown in this model of focal ischaemia, the infarct size is not influenced by a moderate variation of blood pressure, it is unlikely that the minor hypertension induced by the treatment with L-NAME explains the neuroprotection. It can be hypothesized that the stimulation of the N-methyl-D-aspartate (NMDA) receptor induced by the ischaemic insult, could raise cytosolic calcium, due largely to influx through the receptor-operated ions channels, and that this calcium influx will activate NO-synthase and lead to an overproduction of NO (Garthwaite, 1991). NO is a free radical which reacts with the oxygen superoxide (O_2^-) to form peroxynitrite anions (ONOO^-) (Blough & Zafriou, 1985). Peroxynitrite could diffuse for several μm before decomposing to form the powerful and cytotoxic oxidants: hydroxyl radical and nitrogen dioxide (Beckman, 1991). Therefore, inhibition of NO synthase might have reduced the ischaemic superoxide-mediated neuronal injury induced by MCA occlusion in the striatum and in the cortex of rats. However, as NMDA antagonists exert few if any protective effects on striatal infarcts induced by focal ischaemia (Park *et al.*, 1988), our results indicate that NO synthase inhibitors may constitute a more efficient treatment than NMDA antagonists, and suggest that the ischaemic NO production may be, at least in part, unrelated to NMDA receptor overstimulation. The above data demonstrate that inhibition of NO synthesis might represent a new pharmacological strategy for neuroprotection in cerebral ischaemia.

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