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SPECIAL REPORT

L-Arginine dilates rat pial arterioles by nitric oxide-dependent mechanisms and increases blood flow during focal cerebral ischaemia

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L-Arginine ($\geqslant 30$ mg kg^{-1}, i.v.), but not D-arginine (300 mg kg^{-1}) administered 5 min after unilateral common carotid/middle cerebral artery occlusion increased regional cerebral blood flow (rCBF) within the dorsolateral ischaemic cortex in spontaneously hypertensive rats. L-Arginine (300 mg kg^{-1}) increased rCBF from 22 ± 2.7 to 33 ± 4% of baseline as measured by laser-Doppler flowmetry. This increase may explain the ability of L-arginine to reduce infarct size following focal cerebral ischaemia, as reported previously. The mechanism appears to be mediated by nitric oxide since topical L-NAME (1 μM), a nitric oxide synthase inhibitor, decreased pial arteriole calibre from 115 ± 2.2 to 106 ± 0.9% of baseline following L-arginine infusion (300 mg kg^{-1}).

Keywords: L-Arginine; nitric oxide; focal cerebral ischaemia; laser-Doppler flowmetry; vasodilatation; cranial window

Introduction Nitric oxide (NO) is synthesized from the amino acid L-arginine by the enzyme NO synthase (Palmer *et al.*, 1988). NO has been proposed as a mediator of endothelium-dependent vasodilatation and relaxes vascular smooth muscle through guanosine 3′:5′-cyclic monophosphate (cyclic GMP)-dependent mechanisms (see Moncada *et al.*, 1991 for review).

Recently, we showed that L-arginine reduces infarct volume in two models of focal cerebral ischaemia (Morikawa *et al.*, 1992). To explain this effect, we hypothesized that L-arginine augments NO production and increases regional cerebral blood flow (rCBF) above the ischaemic threshold. In this paper, we examine the effects of L-arginine infusion on: (i) rCBF within ischaemic tissue using laser-Doppler flowmetry, and on (ii) normal pial vessels after NO synthase inhibition.

Methods *Laser-Doppler flowmetry* Thirty six male spontaneously hypertensive rats (SHR; 280–340 g; Charles River Labs, Wilmington, MA, U.S.A.) were subjected to common carotid artery (CCA)/middle cerebral artery (MCA) occlusion as described previously (Brint *et al.*, 1988). Briefly, anaesthesia was induced and maintained by halothane, 3 and 0.5% respectively, along with 70% nitrous oxide and the balance oxygen in ventilated animals. The MCA was occluded by a metallic clip (Zen clip, Ohwa Tsusho) just distal to the rhinal fissure within 1 min after CCA occlusion.

rCBF was monitored continuously (BPM 403A, TSI Inc.) as described (Koketsu *et al.*, 1992) through a craniotomy over the dorsolateral cortex (4–6 mm lateral, −2 mm to 1 mm rostral to bregma; the transitional zone from severe to mildly ischaemic in this model, Jacewicz *et al.* 1990).

Closed cranial window Twenty male Sprague Dawley (SD) rats (280–330 g; Charles River Labs) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p., plus 10 mg kg^{-1}, i.p. hourly), paralyzed (pancuronium bromide 0.5–1.0 mg, i.v.) and mechanically ventilated with O_2 supplemented room air; end-tidal P_{CO_2} was monitored continuously (Novametrix Medical Systems, Wallingford, CT). Pial vessels were visualized with an intravital microscope (200 × magnification; Leitz, Germany). A window was placed over the left parietal cortex. The space under the window was then filled with artificial CSF (Levasseur *et al.*, 1975) equilibrated at 37°C with a gas containing 10% O_2, 5% CO_2 and the balance nitrogen. Measurements were taken (VIA-100, Boeckler Instruments) after the image was transposed onto a video monitor (Dage MTI Inc., CCD-72 series, Michigan City, IN, U.S.A.).

Arterial blood pressure and blood gases were monitored and rectal temperature was maintained at 37°C in all experiments.

Chemicals L- or D-Arginine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in distilled water and adjusted to pH 7.0 with sodium hydroxide. N^G-nitro-L-arginine methyl ester (L-NAME; Sigma) was dissolved in artificial CSF immediately before use.

Results There were no significant differences in MAP, pHa, plasma glucose, P_{aO_2}, P_{aCO_2} or rectal temperature between treatment groups when rCBF or pial vessel diameter was monitored (data not shown).

CCA/MCA occlusion reduced rCBF by approximately 80% of baseline (Figure 1). L-Arginine, 30 and 300 mg kg^{-1} increased rCBF following occlusion whereas saline, 3 mg kg^{-1} L-arginine, or 300 mg kg^{-1} D-arginine did not. The findings do not appear to depend upon the choice of anaesthetic. When pentobarbitone (65 mg kg^{-1}) was used instead of halothane/nitrous oxide, L-arginine (300 mg kg^{-1}) also increased rCBF from 29 ± 6 to 44 ± 8% ($n = 4$, $P < 0.05$ by paired Student's t test).

L-Arginine (30 and 300 but not 3 mg kg^{-1}) increased pial vessel diameter in SD rats (Figure 2). Topical L-NAME (1 μM) significantly attenuated these responses. L-Arginine (30 mg kg^{-1}, i.v.) dilated pial arterioles when administered to SHR (113 ± 2.6% ($n = 4$); baseline diameter 43 ± 5.9 μm).

Discussion The observed changes in rCBF from below to approximately the ischaemic threshold for infarction in SHR

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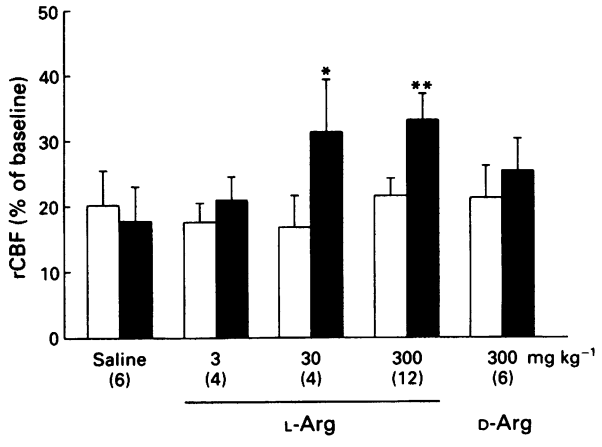

Figure 1 L-Arginine infusion (≥ 30 mg kg^{-1}, i.v.) but not D-arginine increased rCBF in the middle cerebral artery (MCA) region after combined common carotid artery (CCA)/MCA occlusion in SHR. After 15 min of stable rCBF recordings, the CCA/MCA were occluded as described and rCBF measured 2 and 5 min later (open columns). L-or D-Arginine or saline was then administered at a constant rate of 100 µl kg^{-1} min^{-1} for 10 min (Harvard infusion pump, Harvard Bioscience, South Natick, MA, U.S.A.) at the dosages indicated. Post-infusion rCBF, determined at 15 min intervals for the next 105 min, is expressed as the mean of these determinations (solid columns). Data are expressed as percentage of baseline rCBF prior to vessel occlusion (mean ± s.e.mean with number of animals in parentheses). rCBF 2 and 5 min after CCA/MCA occlusion was 20 ± 5.2% for the saline group, and did not differ between treatment groups. $*P<0.05$ and $**P<0.01$ as compared to pre-infusion rCBF, determined by paired Student's t test performed on percentage values.

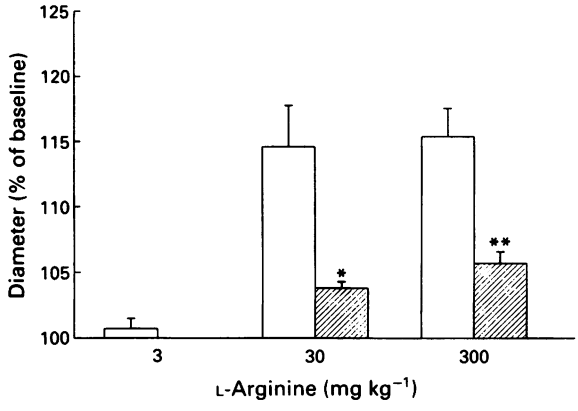

Figure 2 Dilatation of pial vessels following L-arginine infusion was significantly reduced by topical N^G-nitro-L-arginine methyl ester (L-NAME, 1 µM) in SD rats. Baseline diameters were measured after an equilibration period of 30 min through a closed cranial window. Pial vessel diameters (1–3 arterioles per animal) were measured 30 min following L-arginine infusion (3, 30, or 300 mg kg^{-1}, i.v. over 10 min; open columns). In some animals, L-NAME (1 µM) was applied topically 20 min before L-arginine infusion (hatched columns). Data are expressed as percentage of baseline diameter. L-NAME superfusion (1 µM) by itself did not change pial vessel diameter (100 ± 1.2%, $n = 7$). Baseline diameters (mean ± s.e.mean in µm) were 42 ± 2.7 [3 mg kg^{-1}, $n = 3$], 49 ± 3.7 [30 mg kg^{-1}, $n = 5$], and 40 ± 4.1 [300 mg kg^{-1}, $n = 5$] for L-arginine alone; 34 ± 7.0 [30 mg kg^{-1}, $n = 3$] and 40 ± 9.9 [300 mg kg^{-1}, $n = 4$] for L-arginine with L-NAME pretreatment. Error bars denote s.e.mean. $*P<0.05$ and $**P<0.01$, as compared to L-arginine infusion alone by unpaired Student's t test.

(Jacewicz *et al.*, 1992), appear sufficient to explain the previously reported decrease in infarct size following L-arginine infusion. The fact that pial arterioles dilated in response to L-arginine suggests that NO synthase (presumably within the endothelium or within innervating parasympathetic fibres, Nozaki *et al.*, 1992) is not saturated with substrate under resting conditions in the cerebral circulation, and that L-arginine-induced rCBF increases may not be unique to SHR. As indirect evidence, preliminary data indicate that L-arginine infusion also decreases infarct size in SD rats (unpublished observation).

The results described here may seem unexpected in view of published reports showing neurotoxic effects of NO (Dawson *et al.*, 1991) and cytoprotective effects of NO synthase inhibitors in stroke models (Nowicki *et al.*, 1991; Buisson *et al.*, 1992). However, methodological differences between our experiments and those of Nowicki *et al.* and Buisson *et al.* are noteworthy inasmuch as rodents used in their experiments were ventilating spontaneously. Hence, $PaCO_2$ values (not reported by them) were almost certainly high, and baseline blood flows correspondingly high. Alternatively, or in addition, the discrepant results may reflect differences between the effects of NO at the vessel wall versus brain parenchyma *per se* during cerebral ischaemia. These controversies notwithstanding, the findings described here raise the possibility that intravenous administration of L-arginine or other NO precursors may be useful for acutely increasing rCBF during ischaemic strokes in man.

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