

## Intracarotid Infusion of the Nitric Oxide Synthase Inhibitor, L-NMMA, Modestly Decreases Cerebral Blood Flow in Human Subjects

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**Background:** The authors hypothesized that if nitric oxide (NO) was a determinant of background cerebrovascular tone, intracarotid infusion of N<sup>G</sup>-monomethyl-L-arginine (L-NMMA), a

NO synthase (NOS) inhibitor, would decrease cerebral blood flow (CBF) and intracarotid L-arginine would reverse its effect.

**Methods:** In angiographically normal cerebral hemispheres after the initial dose-escalation studies (protocol 1), the authors determined the effect of intracarotid L-NMMA (50 mg/min for 5 min) on CBF and mean arterial pressure (MAP) over time (protocol 2). Changes in CBF and MAP were then determined at baseline, during L-NMMA infusion, and after L-NMMA during L-arginine infusion (protocol 3). To investigate effects of higher arterial blood concentrations of L-NMMA, changes in CBF and MAP were assessed at baseline and after a bolus dose of L-NMMA (250 mg/1 min), and vascular reactivity was tested by intracarotid verapamil (1 mg/min, protocol 4). CBF changes were also assessed during induced hypertension with intravenous phenylephrine (protocol 5).

**Results:** Infusion of L-NMMA (50 mg/min for 5 min, n = 7, protocol 2) increased MAP by 17% ( $86 \pm 8$  to  $100 \pm 11$  mmHg;  $P < 0.0001$ ) and decreased CBF by 20% ( $45 \pm 8$  to  $36 \pm 6$  ml  $100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.005$ ) for 10 min. Intracarotid L-arginine infusion after L-NMMA (protocol 3) reversed the effect of L-NMMA. Bolus L-NMMA (protocol 4) increased MAP by 20% ( $80 \pm 11$  to  $96 \pm 13$  mmHg;  $P < 0.005$ ), but there was no significant decrease in CBF. Intracarotid verapamil increased CBF by 41% ( $44 \pm 8$  to  $62 \pm 9$  ml  $100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.005$ ). Phenylephrine-induced hypertension increased MAP by 20% ( $79 \pm 9$  to  $95 \pm 6$  mmHg;  $P = 0.001$ ) but did not affect CBF.

**Conclusions:** The results suggest that intracarotid L-NMMA modestly decreases CBF, and the background tone of cerebral resistance vessels may be relatively insensitive to NOS inhibition by the intraarterial route. (Key words: Angiography; cerebrovascular resistance, sedation, vasodilator.)

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IT is presently believed that, during early cerebral ischemia, nitric oxide (NO) elaborated by the endothelium mediates vasodilation, thus augmenting collateral blood flow and mitigating against cerebral injury.<sup>1,2</sup> However, during delayed cerebral ischemia, overproduction of NO by neuronal or inducible NO synthase (NOS) can worsen ischemic injury.<sup>3</sup> There is evidence that inhibition of both inducible NOS and neuronal NOS provides neuroprotection.<sup>4,5</sup> In recent years, neuroradiologic interventions such as thrombolysis and angioplasty have been proposed for treating ischemic strokes.<sup>6,7</sup> Technical ad-

vances in interventional radiology now enable selective and safe delivery of the drug directly into cerebral circulation.<sup>8</sup> Such an intraarterial injection limits the initial distribution of the drug to one cerebral hemisphere or less; hence, high arterial concentrations can be achieved at a fraction of a total systemic dose. In theory, intraarterial infusion enables investigation of the cerebrovascular effects of a drug at relative isolation from its systemic effects.<sup>9-11</sup> Thus, an investigation of the role of NO in regulating human cerebral blood flow (CBF) by intracarotid infusion of drugs can help to understand the physiological role of NO in human cerebral circulation and may also influence intraarterial therapies for ischemic stroke.

In most human vascular beds (e.g., skin, pulmonary, or coronary), experiments suggest that NO modulates resting vascular tone and hence regional blood flow.<sup>12-26</sup> However, only few studies so far have investigated the role of NO in regulating resting CBF in human subjects.<sup>27-31</sup> In awake human volunteers, intravenous *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA; 10 mg/kg) decreased internal carotid artery (ICA) blood flow volume by approximately 15% as determined by ultrasonography and by approximately 20% as assessed by positron emission tomography.<sup>27,28</sup> In anesthetized rats, NOS inhibition decreased CBF by as much as 40–60%.<sup>32</sup> In primates, intracarotid infusion of NOS inhibitors decreased CBF by approximately 15%.<sup>33</sup>

In the present study, we assessed the role of NO in regulating vascular tone of minimally sedated resting human subjects by a direct measure of tissue perfusion, the intraarterial <sup>133</sup>Xe washout technique. Changes in resting CBF were assessed after intracarotid infusion of L-NMMA. All three isoforms of NOS—endothelial, inducible NOS, and neuronal NOS—can be competitively inhibited by L-NMMA. We hypothesized that if NO played a significant role in modulating background vascular tone, intracarotid infusion of L-NMMA would decrease CBF, and the effect of L-NMMA would be reversed by intracarotid infusion of a NO agonist, L-arginine.

## Materials and Methods

The study was approved by the Institutional Review Board for research on human subjects. Informed consent was obtained from participating subjects (adults of both sexes) who were undergoing cerebral angiographic pro-

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cedures. Subjects participating in this study were neurologically and medically stable. There was no history of intracranial hemorrhage within 6 weeks before enrollment in the study. All studies were conducted contralateral to the vascular pathology in angiographically normal cerebral vessels. No patient had any evidence of increased intracranial pressure. Patients fasted overnight and received nimodipine (30 mg orally) as a part of their preoperative preparation for the clinical procedure. On arrival in the angiography suite, standard anesthetic monitors were applied. Intravenous sedation (fentanyl/midazolam) with supplemental propofol was titrated to render the patient comfortable but easily arousable for neurologic testing. With fluoroscopic guidance, a 7.0-French coaxial catheter was placed in the cervical ICA through a 7.5-French femoral introducer sheath.<sup>34</sup> Systemic (femoral artery) and ICA pressures were measured relative to the right atrium with strain gauge pressure transducers (Transpac; Abbott Critical Care, North Chicago, IL), displayed in real time on a monitor (Merlin; Hewlett-Packard, Waltham, MA), and digitally recorded with a MacLab system (AD Instruments, Adelaide, Australia).

### Cerebral Blood Flow Measurements

Cerebral blood flow was determined by the intracarotid <sup>133</sup>Xe injection technique. Briefly, the CBF probes are tungsten-collimated (30 × 20 mm) cadmium telluride scintillation detectors from a commercial CBF collection system (Carolina Medical, King, NC). Placement of two detectors over the middle cerebral artery (MCA) distribution was aided by contrast injection during fluoroscopy. A compact bolus dose of <sup>133</sup>Xe in saline (1–2 mCi in 0.5 ml) was injected through the coaxial catheter, and washout was recorded during stable physiological conditions for at least 1.5 min. CBF was calculated using the initial slope method using data collected between 20 and 80 s of tracer washout, which gives a value weighted toward gray matter.<sup>35,36</sup> Washout curves were individually inspected for artifact and curve fit. Mean systemic (femoral mean arterial pressure [MAP]) and ICA pressures as well as an arterial blood sample for determination of arterial carbon dioxide partial pressure and hematocrit were obtained concurrently with each CBF measurement. Cerebrovascular resistance was calculated by dividing ICA pressure by CBF.

### Drug Infusion Protocols

Five drug infusion protocols were used. The first protocol primarily addressed the safety issues with increas-

ing doses of L-NMMA and L-arginine. The second protocol determined the duration of effect after intracarotid L-NMMA infusion. The third protocol assessed reversal of L-NMMA effects by L-arginine. The fourth protocol used a bolus injection of L-NMMA to assess the effect on resting cerebral blood flow of higher arterial blood concentrations of the drug. The fifth protocol investigated the effect of induced hypertension on CBF by an intravenous injection of phenylephrine sufficient to increase MAP by approximately 20%.

**Protocol 1: Initial Dose Escalation Studies.** Initial feasibility studies were undertaken in patients who received increasing doses of L-arginine (R-Gen 10; Pharmacia Inc, Clayton, NJ) or L-NMMA (Clinalfa AG, Switzerland). MCA flow velocity by transcranial Doppler (TCD; Pioneer 2000, Nicolet, Golden, CO), and electroencephalogram (Neurotrac II; Moberg Medical Inc., Ambler, PA) was monitored in these patients. Baseline TCD, electroencephalogram, and CBF were determined. The infusion dose of the drug was increased every 3 min over the next 10–15 min during continuous TCD and electroencephalogram monitoring until the highest target dose of L-NMMA or L-arginine had been reached. CBF was determined for the highest dose. The infusion dose range for L-arginine was 0.17–150 mg/min. For L-NMMA, the dose range was 0.08–7 mg/min (a total maximum dose of 35 mg was reached). We planned additional  $^{133}\text{Xe}$  measurements in the event of any clinically significant changes in MCA flow velocity, neurologic state, or electroencephalogram.

**Protocol 2: Continuous Infusion of L-NMMA.** This protocol generated four sets of CBF and physiological data: baseline, during ICA L-NMMA infusion, 5 min after infusion, and 10 min after infusion. After baseline measurements of CBF and hemodynamic parameters were recorded, L-NMMA was infused at 50 mg/min. Three minutes after L-NMMA infusion, the bolus dose of  $^{133}\text{Xe}$  was injected into the carotid artery. A small bolus dose of L-NMMA was used to prime the coaxial catheter after  $^{133}\text{Xe}$  injection. Tracer washout was recorded for the next 90 s, during which intracarotid L-NMMA infusion was resumed. The hemodynamic parameters were determined at the end of tracer washout. A total dose of 250 mg of L-NMMA was infused over 5 min. L-NMMA infusion was replaced by normal saline. CBF was determined again 10 and 15 min after the start of the experiment. The corresponding hemodynamic parameters were recorded after 90 s of tracer washout.

**Protocol 3: Sequential Infusion of L-NMMA and L-Arginine.** After baseline CBF and hemodynamic measurements were recorded, L-NMMA was infused at 50 mg/min for 3 min. Intraarterial  $^{133}\text{Xe}$  was injected and L-NMMA infusion resumed. Tracer washout was recorded over the next 90 s, and at that time the hemodynamic parameters were determined. The L-NMMA infusion was replaced by L-arginine infusion at a rate of 150 mg/min. After 3 min of L-arginine infusion,  $^{133}\text{Xe}$  was injected, and CBF was determined over the next 90 s. During tracer washout, L-arginine infusion was resumed. Hemodynamic parameters were recorded 90 s after  $^{133}\text{Xe}$  injection. A total of 750 mg of L-arginine was infused in approximately 4.5 min. Thus, with this infusion protocol, three sets of CBF and hemodynamic measurements were obtained: at baseline and during L-NMMA and L-arginine infusion.

**Protocol 4: Bolus L-NMMA Injection.** Three sets of CBF and physiological measurements were undertaken with protocol 4. After baseline measurements were recorded, 250 mg of L-NMMA was infused into the carotid artery over 1 min. Intraarterial  $^{133}\text{Xe}$  was injected 2 min after completion of the infusion, and tracer washout was recorded for the next 90 s. Hemodynamic parameters were recorded at the end of tracer washout. Five minutes after the start of L-NMMA infusion, a bolus dose of intraarterial verapamil (1 mg/min) was infused into the carotid artery for 3 min, followed by a bolus  $^{133}\text{Xe}$  injection. Infusion of verapamil was resumed after  $^{133}\text{Xe}$  injection. Tracer washout was recorded over the next 90 s, at which time the hemodynamic parameters were determined. Verapamil was given to demonstrate intact vascular reactivity of the studied vascular territory. This protocol generated three sets of CBF and physiological data: at baseline, 2 min after bolus L-NMMA injection, and during infusion of verapamil.

**Protocol 5: Induced Hypertension Group.** To control for the increases in systemic arterial pressure that were observed with L-NMMA infusion, two sets of CBF and physiological measurements were undertaken. After baseline measurements were recorded during saline infusion, intravenous phenylephrine (10–40  $\mu\text{g}/\text{min}$ ) was infused to increase MAP by approximately 20%. This increase in MAP corresponded to the increase seen with L-NMMA. Corresponding CBF and physiological measurements were made after 3 min during phenylephrine infusion. Phenylephrine infusion was continued during tracer washout.

**Table 1. Effect of Continuous Intracarotid Infusion of L-NMMA**

	Baseline	L-NMMA	5 min Postinfusion	10 min Postinfusion
Hematocrit (%)	33 ± 6	33 ± 6	32 ± 5	33 ± 6
Paco <sub>2</sub> (mmHg)	41 ± 5	43 ± 6	41 ± 6	42 ± 5
Heart rate (beats/min)	64 ± 13	58 ± 15	58 ± 16*	60 ± 13
MAP (mmHg)	86 ± 8	100 ± 11*	102 ± 11*	102 ± 9*
P <sub>ica</sub> (mmHg)	85 ± 7	100 ± 9*	102 ± 11*	101 ± 9*
CBF (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )	45 ± 8	36 ± 6*	37 ± 3*	37 ± 5*
CVR (mmHg · ml <sup>-1</sup> · 100 g <sup>-1</sup> · min <sup>-1</sup> )	1.9 ± 0.4	2.9 ± 0.5*	2.8 ± 0.3*	2.8 ± 0.4*

n = 7, protocol 2.

\* Significant *post hoc* test results different from baseline.

Paco<sub>2</sub> = partial pressure of carbon dioxide and arterial blood; MAP = mean arterial pressure; P<sub>ica</sub> = internal carotid artery pressure; CBF = cerebral blood flow; CVR = cerebrovascular resistance.

### Statistical Analysis

The data are presented as mean ± 1 SD. Statistical evaluations of continuous variables were undertaken by repeated-measures analysis of variance, and *post hoc* testing for multiple comparisons was conducted with the Bonferroni Dunn test. *P* < 0.05 was considered significant.

## Results

A total of 38 studies were conducted on 32 adult subjects (mean age, 42 ± 13 yr; range, 21–67 yr; 13 men, 19 women). Of the 32 patients, 23 harbored hemispheric cerebral arteriovenous malformations, one had vertebral artery fistula, four had previously treated cerebral aneurysms, two had intracranial tumors, and two had no demonstrable intracranial pathologies. Six patients had a history of intracranial bleeds ranging from 2 months to 7 yr before the study. Baseline CBF was comparable in patients with vascular and nonvascular pathologies, as well as between patients with and without cerebral arteriovenous malformations. Data from two studies were lost because of technical reasons. In one patient there was accidental dislodgment of the detectors, and in the second there was mechanical failure of the infusion pump. Data from the remaining 36 studies were available for analysis. Five patients were enrolled for research on more than one occasion. One patient was enrolled three times, and four were enrolled twice. However, no patient was assigned to the same drug infusion protocol. During the studies, the physiological conditions, namely, the arterial carbon dioxide partial pressure and hematocrit, did not show any change.

### Protocol 1: Dose-Escalation Studies

Initial feasibility studies were conducted with L-arginine (n = 5) and L-NMMA (n = 6). The objective was to assess the safety of intracarotid drug infusion and arrive at a target dose that would result in an approximate 20% decrease in CBF after L-NMMA. The L-NMMA group had five women and one man whose mean age, weight, and height were 46 ± 15 yr, 163 ± 5 cm, and 67 ± 13 kg, respectively. The L-arginine group had four men and one woman, whose mean age, weight, and height were 45 ± 15 yr, 170 ± 14 cm, and 80 ± 14 kg, respectively. One patient in each group had history of hypertension and remote intracranial hemorrhage. Assuming an ICA blood flow of 200 ml/min, the calculated molar concentration of L-arginine (molecular weight 211) in the ICA blood ranged from 4 to 3,500 μM. In the case of L-NMMA (molecular weight 248), the dose range of the drug generated an estimated molar concentration of 2–140 μM in the ICA blood. None of the patients who received L-arginine or L-NMMA showed any neurologic, electroencephalogram, or TCD changes. Hemodynamic parameters were not affected by either drug at any dose.

### Protocol 2: Continuous L-NMMA Infusion Protocol

Seven subjects (five women and two men) who enrolled in this study segment had a mean age, weight, and height of 44 ± 18 yr, 168 ± 13 cm, and 85 ± 32 kg, respectively. One patient had history of remote intracranial hemorrhage. Infusion of L-NMMA at 50 mg/min into the carotid artery was associated with a decrease in CBF from baseline (45 ± 8 to 36 ± 6 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>; *P* = 0.0007; n = 7). The estimated ICA blood concentration at this infusion rate was 1 × 10<sup>3</sup> μM. The CBF remained low at 5 min (37 ± 3 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>) and 10 min (37 ± 5 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>) after L-NMMA



## INTRACAROTID L-NMMA AND CBF

**Table 2. Effect of Sequential Intracarotid Administration of L-NMMA and L-Arginine**

	Baseline	L-NMMA	L-Arginine
Hematocrit (%)	34 ± 5	33 ± 5	33 ± 5
Paco <sub>2</sub> (mmHg)	48 ± 8	50 ± 10	49 ± 9
Heart rate (beats/min)	76 ± 16	71 ± 18*	73 ± 13
MAP (mmHg)	73 ± 10	82 ± 12*	77 ± 12
P <sub>ica</sub> (mmHg)	68 ± 14	80 ± 12*	74 ± 12
CBF (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )	55 ± 20	48 ± 23*	52 ± 20
CVR (mmHg · ml <sup>-1</sup> · 100 g <sup>-1</sup> · min <sup>-1</sup> )	1.4 ± 0.4	1.9 ± 0.8*	1.6 ± 0.5†

n = 6, protocol 3.

Significant *post hoc* test results: \* different from baseline; † different from L-NMMA.

Paco<sub>2</sub> = arterial carbon dioxide tension; MAP = mean arterial pressure; P<sub>ica</sub> = internal carotid artery pressure; CBF = cerebral blood flow; CVR = cerebrovascular resistance.

infusion. MAP increased from 86 ± 8 mmHg at baseline to 100 ± 11 mmHg during L-NMMA infusion ( $P < 0.0001$  compared with baseline). Compared with baseline it remained high at 5 min (102 ± 11 mmHg;  $P < 0.0001$ ) and at 10 min (102 ± 9 mmHg;  $P < 0.0001$ ) after L-NMMA infusion (table 1).

#### Protocol 3: Sequential Infusion of L-NMMA and L-Arginine

Six patients, three men and three women, were enrolled for the sequential infusion arm of the study. The mean age, weight, and height of the patients was 45 ± 7 yr, 163 ± 6 cm, and 77 ± 20 kg, respectively. Three patients in this group indicated a remote history of intracranial hemorrhage. One patient was receiving treatment for essential hypertension. Infusion of L-NMMA at 50 mg/min increased MAP (73 ± 10 to 82 ± 12 mmHg;  $P < 0.002$ ; n = 6) and decreased CBF (55 ± 20 to 48 ± 23 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.005$ ). Infusion of L-arginine after L-NMMA resulted in an increase in CBF (48 ± 23 to 52 ± 20 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>) and a decrease in MAP (82 ± 12 to 77 ± 12 mmHg) that showed a strong trend toward significance. As with the continuous L-NMMA infusion protocol, the estimated ICA blood concentration at this infusion rate was  $1 \times 10^3 \mu\text{M}$ . Infusion of L-arginine reversed the effects of L-NMMA. The difference in CBF and MAP during L-arginine infusion and the baseline values was not significant (table 2 and fig. 1).

#### Protocol 4: Bolus Injection of L-NMMA

Seven patients (five women and two men) were enrolled in this arm of the study. The mean age, weight, and height of the patients were 35 ± 13 yr, 163 ± 5 cm,

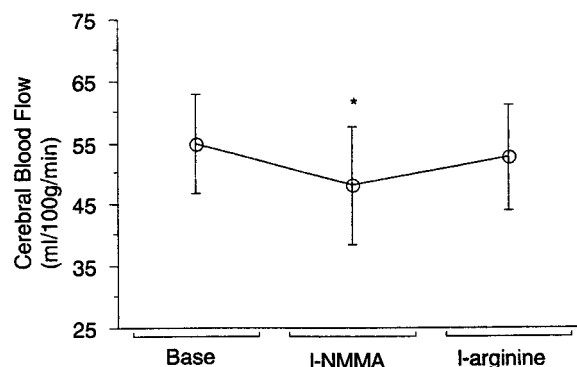
and 67 ± 15 kg, respectively. One patient had a remote history of hemorrhage. Bolus injection of L-NMMA resulted in a MAP increase from baseline (80 ± 11 to 96 ± 13 mmHg;  $P < 0.002$ ; n = 7). The estimated ICA blood concentration was  $5 \times 10^3 \mu\text{M}$ . A trend for a decrease in CBF with L-NMMA was observed (51 ± 16 to 44 ± 8 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.2$ ; table 3). Infusion of verapamil after bolus L-NMMA infusion decreased MAP (96 ± 13 to 84 ± 9 mmHg;  $P < 0.002$ ) and increased CBF (from 44 ± 8 to 62 ± 9 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.005$ ).

#### Induced Hypertension Group

Five patients (four women and one man) were enrolled in this arm of the study. The mean age, weight, and height of the patients were 38 ± 10 yr, 164 ± 8 cm, and 76 ± 18 kg, respectively. One patient had remote history of hemorrhage and one was receiving antihypertensive treatment. Intravenous phenylephrine increased MAP by 79 ± 8 to 95 ± 6 mmHg ( $P < 0.001$ ; n = 5). Cerebrovascular resistance increased during induced hypertension (1.6 ± 0.5 to 2.2 ± 0.6 mmHg per ml · 100 g<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.03$ ). A nonsignificant trend toward a decrease in CBF was observed during phenylephrine infusion (52 ± 16 to 46 ± 16 ml · 100 g<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.2$ ; table 4).

## Discussion

The primary finding of this study was that intracarotid infusion of L-NMMA for 5 min in angiographically and functionally normal cerebral hemispheres decreased CBF by 13–20%. However, this required an intracarotid dose of L-NMMA that was sufficient to increase the mean



**Fig. 1.** Changes in cerebral blood flow after infusion of L-NMMA (50 mg/min) followed immediately by infusion of L-arginine (150 mg/min, protocol 3). The asterisk represents a significant difference from baseline and L-arginine.

**Table 3. Effect of Intracarotid Bolus Administration of L-NMMA Followed by Verapamil Challenge**

	Baseline	L-NMMA	Verapamil
Hematocrit (%)	30 ± 5	30 ± 5	30 ± 5
Paco <sub>2</sub> (mmHg)	48 ± 3	47 ± 3	47 ± 2
Heart rate (beats/min)	72 ± 14	66 ± 10	70 ± 11
MAP (mmHg)	80 ± 11	96 ± 13*	84 ± 9†
P <sub>ica</sub> (mmHg)	79 ± 11	96 ± 13*	83 ± 9†
CBF (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )	51 ± 16	44 ± 8	62 ± 9†
CVR (mmHg · ml <sup>-1</sup> · 100 g <sup>-1</sup> · min <sup>-1</sup> )	1.7 ± 0.7	2.3 ± 0.7	1.3 ± 0.2†

n = 7, protocol 4.

Significant post hoc test results: \* different from baseline; † different from L-NMMA.

Paco<sub>2</sub> = arterial carbon dioxide tension; MAP = mean arterial pressure; P<sub>ica</sub> = internal carotid artery pressure; CBF = cerebral blood flow; CVR = cerebrovascular resistance.

blood pressure by 14–19% on recirculation of the drug. The decrease in CBF lasted for at least 10 min, was reversed by infusion of L-arginine, and is similar to that reported in literature after intravenous injection of L-NMMA.<sup>27,28</sup> The decrease in CBF after intraarterial L-NMMA is much less than the decrease reported in literature for skin or coronary blood flows.<sup>14,22,23,37</sup> Our results suggest that intracarotid L-NMMA modestly decreases CBF and that the background tone of cerebral resistance vessels may be relatively insensitive to NOS inhibition by the intraarterial route.

This intracarotid drug challenge study was undertaken to investigate the effects of L-NMMA on cerebral circulation in relative isolation from its systemic side effects. After intracarotid injection, the drug is distributed in the ICA blood flow, approximately 200 ml/min.<sup>38</sup> In contrast, after intravenous injection, the injected drug is distributed in the entire cardiac output, approximately 5,000 ml/min. In simple pharmacokinetic terms, arterial blood concentration is approximately 25-fold greater after intracarotid injection than after intravenous injection. Thompson *et al.*<sup>33</sup> demonstrated that an arterial blood concentration of L-NMMA of approximately 1–10  $\mu$ M with intracarotid infusion causes a 15% decrease in CBF in anesthetized baboons without any increase in MAP. During the initial feasibility studies, we had primarily targeted such a dose range of the drug (2–140  $\mu$ M); however, we observed no consistent decrease in CBF. We therefore increased our intracarotid doses to approach those delivered through the intravenous route in other studies.<sup>17,28</sup> We estimated that our doses would generate adequate pharmacologic cerebral arterial blood concentrations, *i.e.*, in the 1,000–5,000- $\mu$ M range, at least two orders of magnitude higher than those used by Thompson *et al.* in nonhuman primates.<sup>33</sup>

Large intracarotid doses of L-NMMA used with proto-

cols 2, 3, and 4 increased MAP during recirculation of the drug. However, we did not treat the increase in MAP after L-NMMA infusion. To assess the effect of increased systemic blood pressure, we infused intravenous phenylephrine in a separate group of patients (induced hypertension group). In the induced hypertension group, increase in MAP by intravenous phenylephrine was associated with an increase in cerebral vascular resistance and a nonsignificant trend toward a decrease in CBF. Thus, during our experiments, cerebral autoregulatory vasoconstriction did not seem to be impaired by sedation, nimodipine premedication, or the use of radiocontrast required for the clinical angiographic procedure.

The effects of intracarotid L-NMMA in human subjects have not been described. During dose-escalation studies with L-arginine and L-NMMA, we used TCD flow measurements over the MCA and electroencephalogram measurements to provide us with an early warning of any decrease in cerebral perfusion. However, we did not observe any such changes either in the TCD or electroencephalogram. By stopping sedation approximately 10 min before the experiment, we were able to communicate with the patient. This enabled us to conduct gross neurologic examination when needed and permitted the patient to describe any sensory changes. Therefore, for subsequent protocols, we discontinued electroencephalogram monitoring. With regard to discontinuing the TCD measurements, White *et al.*<sup>28</sup> reported that reduction in ICA flow volumes after intravenous L-NMMA may not be associated with corresponding changes in flow velocity measured by the TCD. White *et al.* have suggested that MCA constriction could mask the reduction in CBF caused by L-NMMA when measured by the TCD.<sup>28</sup> Other studies also suggest that MCA diameter may be sensitive to NO.<sup>31</sup> If there are changes in MCA diameter,

**Table 4. Pressure (Induced Hypertension) Control Group**

	Baseline	Intravenous PE
Hematocrit (%)	35 ± 3	35 ± 2
Paco <sub>2</sub> (mmHg)	49 ± 4	46 ± 3
Heart rate (beats/min)	69 ± 7	63 ± 6*
MAP (mmHg)	79 ± 8	95 ± 6*
P <sub>ica</sub> (mmHg)	79 ± 9	95 ± 7*
CBF (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )	52 ± 16	46 ± 16
CVR (mmHg · ml <sup>-1</sup> · 100 g <sup>-1</sup> · min <sup>-1</sup> )	1.6 ± 0.5	2.2 ± 0.6*

n = 5.

\* Significant differences from baseline.

Paco<sub>2</sub> = arterial carbon dioxide tension; PE = phenylephrine; MAP = mean arterial pressure; P<sub>ica</sub> = internal carotid artery pressure; CBF = cerebral blood flow; CVR = cerebrovascular resistance.

then TCD flow velocity measurements will not be reliable indicator of CBF.

Continuous infusion of L-NMMA at 50 mg/min (protocol 2) decreased CBF by approximately 20% for at least 10 min. White *et al.*<sup>28</sup> observed that intravenous L-NMMA (10 mg/kg) resulted in a peak decrease in ICA flow volume of 15% within 5 min that lasted for at least 45 min after cessation of infusion. A lesser decrease in CBF (≈13%) was evident after L-NMMA (50 mg/min) with the sequential infusion protocol (protocol 3). As shown in tables 1 and 2, subjects who received sequential infusion of L-NMMA and L-arginine demonstrated a higher mean arterial carbon dioxide partial pressure and lower mean blood pressure, which may suggest a greater degree of background sedation. The specific involvement of NO in maintaining cerebral vascular tone was demonstrated by an infusion of L-arginine after L-NMMA (protocol 3) that reversed the effect of L-NMMA on CBF and MAP. Recent evidence suggests that the specific reversal of the effects of arginine analogs by L-arginine may not only be caused by the modulation of the cyclic guanosine monophosphate pathway, but could also be mediated by adenosine triphosphate-sensitive potassium channels.<sup>39</sup> Intracarotid infusion of L-arginine during dose-escalation studies did not affect CBF. Intracarotid infusion of L-NMMA (protocols 2 and 3) resulted in a 17% and 14% increase in MAP, respectively. The increase in MAP after intracarotid L-NMMA was very similar to the increase in MAP observed after intravenous administration reported by several other groups.<sup>17,18,28</sup>

Bolus injection of 250 mg of L-NMMA (protocol 4) should have theoretically generated a fivefold higher arterial blood concentration as compared with continuous or sequential infusion protocols, yet it failed to cause a more profound decrease in CBF when compared with

continuous infusion. CBF was determined at 2 min after bolus L-NMMA injection, and tracer washout was recorded over the next 90 s. Thus, CBF was determined between 2 and 3.5 min of L-NMMA injection. In humans, NOS inhibition with intravenous L-NMMA results in a rapid dose-dependent decrease in CBF that is evident within 5 min of drug infusion and lasts for at least 15–45 min.<sup>28</sup> In animals, although a much longer period of time is required to completely inhibit NOS,<sup>32,40,41</sup> near-maximal reduction in CBF is evident within 10 min.<sup>32</sup> Alternatively, it is possible that lack of dose-response relationship after bolus L-NMMA may reflect a true maximal pharmacologic effect or saturation of the L-arginine transport system into the endothelium.<sup>42,43</sup> Infusion of verapamil after bolus L-NMMA administration increased CBF by approximately 41%. This increase in CBF is similar to the increase observed after superselective intraarterial verapamil in humans in our previous work.<sup>44</sup> This observation suggests that the vascular territories studied had relatively normal vascular reactivity and that bolus L-NMMA infusion does not affect vasodilation caused by calcium channel blockade.

In contrast to coronary and brachial circulations, the human cerebral circulation may be relatively resistant to NOS inhibition by the intraarterial route. In the brachial circulation, for example, L-NMMA can decrease regional blood flow by 50%.<sup>37</sup> A 20–30% reduction in blood flow has been reported in the coronary circulation.<sup>14,22,23</sup> However, in humans, the reduction of CBF with L-NMMA seems to be on the order of 13–20%, as suggested by this study and previous data.<sup>27,28</sup> The relative insensitivity of CBF to NOS inhibition can also be illustrated by the data provided by White *et al.*<sup>28</sup> On the basis of their data, it is possible to infer that intravenous L-NMMA (10 mg/kg) can result in a threefold greater reduction in the external than in the ICA blood flow volume.

In the present study we did not directly measure changes in NOS activity after intracarotid L-NMMA. Stamler *et al.*<sup>17</sup> observed a 65% decrease in NOS activity after intravenous L-NMMA (0.01–1 mg · kg<sup>-1</sup> · min<sup>-1</sup>) in human subjects. In rats, for example, doses of NOS inhibitors that result in approximately 20% increase in MAP are associated with approximately 50% decrease in NOS activity.<sup>40,45</sup> Similarities between the increase in blood pressure in our study and those using intravenous L-NMMA infusion suggest that hypertension in our study was the result of systemic effects and not a direct effect of the drug on the central nervous system, although the latter possibility cannot be completely discounted. In the doses used in our study, a significant decrease in

NOS activity should have been achieved, as evidenced by the increase in MAP. Nevertheless, there was only a modest reduction in CBF. Because of primarily safety concerns, our goal was to use a dose of L-NMMA that would decrease CBF or increase MAP by 15–20% and not completely inhibit NOS.

There are limitations of conducting clinical studies during cerebral angiography. Such studies are not practical or feasible in healthy human subjects. Although we investigated human subjects who harbored intracranial pathologies, none of these patients had evidence of increased intracranial pressure or were neurologically unstable. We investigated the contralateral cerebral hemisphere that was angiographically and functionally normal. The increases in CBF after intraarterial verapamil suggest that pharmacologic reactivity to calcium channel blockade was intact in these vascular territories. As a part of the clinical protocol, we were required to give oral nimodipine premedication and sedation. All sedation was stopped approximately 10 min before the start of the experiment, and the patients were easily arousable by verbal commands. Nonetheless, the effect of residual sedation cannot completely be ruled out. Nimodipine is empirically given at Columbia University, New York, New York, to prevent catheter-induced vasospasm. Both nimodipine and sedation could influence baseline CBF. However, the baseline CBF in all groups was within the normal range. Ongoing primate studies<sup>46</sup> suggest that vascular responses in our human clinical studies are similar to those of anesthetized healthy baboons. Such parallel human and higher primate studies are ideal to investigate the confounding effects of diseases and drugs.

Our results suggest that intracarotid injection of the NOS inhibitor L-NMMA decreases CBF of minimally sedated resting human subjects by as much as 20% and that, compared with other regional vascular beds, human cerebral circulation might be relatively insensitive to NOS inhibition by the intraarterial route. Because we used a nonspecific NOS inhibitor in our studies, the relative importance of neuronal *versus* endothelial NOS in regulating resting CBF remains to be investigated. Further studies with selective neuronal NOS inhibitors are required to address this issue.

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