Nitric Oxide Synthase Inhibition in Humans Reduces Cerebral Blood Flow but Not the Hyperemic Response to Hypercapnia

Richard P. White, MRCP; Colin Deane, PhD; Patrick Vallance, MD; Hugh S. Markus, DM

Background and Purpose—Animal studies suggest that nitric oxide (NO) is important in basal cerebral blood flow (CBF) regulation and that it may mediate the vasodilatory response to carbon dioxide. We investigated its role in the human circulation using the NO synthase inhibitor N<sup>g</sup>-monomethyl-L-arginine (L-NMMA).

Methods—L-NMMA was administered as an intravenous bolus at three doses (1, 3, and 10 mg/kg). CBF was assessed by color velocity ultrasonic imaging of internal and common carotid artery volume flow (ICA flow and CCA flow) and transcranial Doppler ultrasound measurement of middle cerebral artery flow velocity (MCAv). The pressor effect of L-NMMA was controlled for by comparison with noradrenaline titrated to effect an equivalent blood pressure elevation.

Results—L-NMMA produced a dose-dependent reduction in basal mean±SD CCA flow from 415.2±51.9 to 294±125.4 mL/min (at 10 mg/kg) and ICA flow from 268.8±59.4 to 226.2±72.6 mL/min (P<.005 and P<.05, respectively, comparing areas under the dose-response curve). This was reversed by L-arginine. Mean±SD systemic blood pressure rose from 85.2±6.4 to 100.8±9.6 mm Hg (P<.01). There was no significant reduction in MCAv. There was no significant change in the CBF response to either 6% or 8% carbon dioxide after L-NMMA. Noradrenaline produced a lesser fall in basal CCA flow (12.0%) but had a similar effect on the hypercapnic response.

Conclusions—Basal NO release is important in controlling human CBF, but intravenously administered L-NMMA does not inhibit the hypercapnic hyperemic response in humans. The discrepancy between CBF and MCAv after L-NMMA administration is consistent with MCA vasoconstriction. Neuronal NO synthase inhibition may be protective in stroke. However, our results suggest that nonselective NO synthase inhibitors such as L-NMMA should be used with caution because they reduce CBF. (Stroke. 1998;29:467-472.)

Key Words: cerebrovascular circulation • nitric oxide • noradrenaline • ultrasonography, Doppler
studied basal and hyperemic responses after noradrenaline administration at an equivalent pressor dose.

Subjects and Methods

Three separate studies, each on 6 subjects, were performed to determine (1) the effect of different doses of L-NMMA on carotid artery flow, (2) the effect of L-NMMA on the hyperemic response to hypercapnia, and (3) the effect of noradrenaline (titrated to result in a rise in blood pressure similar to that of L-NMMA) on both basal carotid artery flow and the hyperemic response to hypercapnia. Therefore, a total of 18 studies were performed in 13 healthy volunteers (4 women), mean±SD age 27.9±3.3 years and weight 73.7±16.5 kg. In 5 individuals, two studies were performed, but at least 1 month was allowed between the two studies. All subjects had abstained from alcohol for the preceding 24 hours, but all had abstained for the preceding 24 hours. The project was approved by the King’s College Hospital Ethics Committee. Informed written consent was obtained from all subjects.

Ultrasonic quantification of right CCA and ICA blood volume flow was performed using a color velocity imaging system (Philips P700 CVI-Q). This is a color flow imaging method that combines M-mode imaging with time-domain processing for simultaneous determination of flow velocity and functional vessel diameter, from which effective flow volume is calculated as an integrative function. In vitro validation with flow phantoms shows good correlation with volume flows within the physiological range. We have previously determined the reproducibility of flow measurements in 18 healthy subjects. Mean±SD percent variation between repeated measurements was 6.3±6.9% for the CCA and 9.7±9.8% for the ICA. MCAv was measured with transcranial Doppler ultrasonography via the transtemporal route using a 2-MHz probe. During all studies, end-tidal CO2 was continuously recorded (Datex Normocap 200). For the hypercapnia study, CO2 was administered via a face mask with both inspiratory and expiratory limbs protected by one-way valves. During all studies, MAP and pulse rate were monitored using an automated blood pressure cuff (Omega 1400, In-Vivo Laboratories plc), and for each reading a mean of three measurements was taken. All subjects were studied in a supine position with the head elevated on a pillow, after a 15-minute rest period.

Basal CBF Study

The effect of L-NMMA at three doses (1, 3, and 10 mg/kg) on CCA volume flow (CCA flow) and ICA volume flow (ICA flow), and MCAv was determined. L-NMMA was obtained from Glaxo Wellcome plc, and its purity was 99.8%. During a 20-minute control interval, two sets of volume flow measurements (each the mean of two readings) were taken. Each dose of L-NMMA (clinical grade, supplied by Glaxo Wellcome and diluted in 0.9% NaCl) was administered as a bolus injection through an intravenous cannula, and measurements were repeated at 5-minute intervals up to 25 minutes after dose. Following the 10 mg/kg dose, further measurements were taken at 35 and 45 minutes after dose.

Hypercapnic Hyperemia Study

After the dose-ranging basal CBF study, two doses of L-NMMA were used in this study (3 and 10 mg/kg). Because of time constraints during the administration of CO2 and the more difficult acquisition during the increased respiratory excursions, only CCA and not ICA measurements were made. MCAv was continuously recorded. During a 20-minute control interval, two baseline recordings of CCA flow (each the mean of three measurements) were made: breathing room air and then breathing 6% and 8% CO2 in air. In each case, readings were taken after end-tidal CO2 had stabilized and always >2 minutes after CO2 had been started. After a 20-minute rest period, measurements were repeated for breathing room air, before and 5 minutes after administration of 3 mg/kg L-NMMA, and then after 6% and 8% CO2. This process was repeated for 10 mg/kg L-NMMA, and an additional time point at 45 minutes after dose was included. In 4 subjects, the specificity of the response was assessed by administration of l-arginine in molar excess at a dose of 30 mg/kg (Clinalfa) immediately following the 45-minute post–10 mg/kg L-NMMA run, and repeating the measurements taken at rest and after 6% and 8% CO2.

Noradrenaline Study

A protocol similar to that for hypercapnic hyperemia was used. Intravenous noradrenaline (Sanofi Winthrop, diluted in 5% dextrose to 10 μg/mL) was titrated against resting MAP to effect a 15 to 20 mm Hg elevation (equivalent to that observed with 10 mg/kg L-NMMA). Before noradrenaline administration, CCA flow measurements were taken for breathing room air and for 6% and 8% CO2. After noradrenaline administration, once the desired MAP was reached, baseline and hypercapnia measurements were repeated. MCAv was continuously recorded.

Statistical Analysis

For the basal study, the dose-response relationships for CCA flow, ICA flow, MCAv, MAP, and pulse with L-NMMA were compared with the control period by analysis of the AUC over the first 20 minutes after dose, followed by paired t tests (baseline versus after dose). For the hypercapnia and noradrenaline studies, the increase in CCA flow is expressed as an absolute value and also as a percentage of increase relative to normocapnia after 8% CO2, which produces a maximal vasodilatory response in humans. Breathing 6% CO2 produces a submaximal response; therefore, CCA flow values are expressed per kilopascal of increase in end-tidal CO2. Paired t tests were used to compare the response after L-NMMA and noradrenaline. In the 4 subjects who received l-arginine, the hypercapnic hyperemic responses were compared with the 45-minute post–10 mg/kg dose readings. The effects of noradrenaline and L-NMMA on basal and stimulated flow were compared using the unpaired t test.

Results

Basal CBF Study

There was no change in CCA or ICA flow during the control period (CCA 421.8±47.2 and 415.2±51.9 mL/min, P=NS; ICA 278.7±44.4 and 268.8±59.4 mL/min, P=NS). A rapid and dose-dependent reduction in CCA and ICA volume flow after administration of L-NMMA was observed (Fig 1). Mean±SD CCA flow fell from 415.2±51.9 before L-NMMA to 336.5±50.2, 329.8±64.2, and 294.0±56.1 mL/min after 1, 3, and 10 mg/kg, respectively (AUC analysis, P<.005 for all doses). This equated to a maximal reduction of 29.5±6.8%. ICA flow fell from 268.8±59.4 before L-NMMA to 246.7±67.8, 235.8±90.1, and 226.2±72.6 mL/min after 1, 3, and 10 mg/kg, respectively (AUC analysis, P<.05 for all doses). This equated to a maximal reduction of 14.9±21.3%. Results from the AUC analysis are shown in Table 1. There was no significant reduction in MCAv (before L-NMMA,
There was a dose-dependent increase in MAP and reduction in pulse rate (Fig 1 and Table 1) that closely mirrored the changes in CCA flow and ICA flow. The maximal increase in MAP was observed 10 minutes after 10 mg/kg L-NMMA (101.8 ± 9.8 versus 85.2 ± 6.4 mm Hg; AUC analysis, \( P < .01 \)). The pulse rate fell maximally from 59.3 ± 8.5 min⁻¹ before L-NMMA to 44.0 ± 4.6 min⁻¹ at 5 minutes after 10 mg/kg L-NMMA (AUC analysis, \( P < .02 \)). There was no significant change in resting end-tidal CO₂ after L-NMMA administration.

Hypercapnic Hyperemia Study

There was no change in CCA during the control period (CCA, 346.5 ± 28.4 and 340.3 ± 42.9 mL/min, \( P = \text{NS} \)). As for the basal CBF study, there was a dose-dependent reduction in mean ± SD resting CCA flow from 340.3 ± 42.9 to 254.0 ± 36.0 and 225.2 ± 34.1 mL/min after 3 and 10 mg/kg L-NMMA, respectively (\( P < .01 \)) (see Fig 2). There was no significant difference between end-tidal CO₂ levels during rest, 6% and 8% CO₂ in each run of the protocol (mean values: rest 4.88, 6% CO₂ 6.79, 8% CO₂ 8.12 kPa).

The effect of L-NMMA on the vasodilatory response to both 6% and 8% CO₂ are shown in Table 2. There was no significant change in the absolute or percent change in CCA flow after 8% CO₂ or in the percent change divided by end-tidal CO₂ rise for 6% CO₂.

After L-NMMA there was no significant difference in resting MCA\( \text{r} \): before 64.7 ± 13.0, after 3 mg/kg 63.6 ± 11.1, and after 10 mg/kg 63.3 ± 11.8 cm/s. There was no significant change in either 6% or 8% CO₂ MCA\( \text{r} \) reactivity. Mean ± SD reactivity to 6% was 24.9 ± 4.5 before, 24.8 ± 6.6 after 3 mg/kg, and 20.6 ± 4.3%/kPa after 10 mg/kg. Mean ± SD reactivity to 8% was 65.4 ± 21.3 before, 70.9 ± 22.9 after 3 mg/kg, and 70.1 ± 31.4% after 10 mg/kg.

The baseline resting CCA flow was still suppressed at 45 minutes after 10 mg/kg L-NMMA. After l-arginine, baseline and absolute CCA flow responses to hypercapnia returned to pre-L-NMMA values (Table 3) along with the systemic hemodynamic variables of MAP and pulse, confirming the specificity of the effect of L-NMMA.

**TABLE 1.** Mean ± SD Area Under CCA Flow, ICA Flow, MCA\( \text{r} \), MAP, and Pulse Rate Dose-Response Curves for 20-Min Control Period and First 20 Min After Each Dose of L-NMMA

<table>
<thead>
<tr>
<th>Dose</th>
<th>CCA</th>
<th>( P^* )</th>
<th>ICA</th>
<th>( P^* )</th>
<th>MCA( \text{r} )</th>
<th>( P^* )</th>
<th>MAP</th>
<th>( P^* )</th>
<th>Pulse, bpm</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/kg</td>
<td>8370.0 ± 969.0</td>
<td>.000</td>
<td>5748.3 ± 956.1</td>
<td>.000</td>
<td>1288.3 ± 143.6</td>
<td>.000</td>
<td>1683.3 ± 135.2</td>
<td>.000</td>
<td>1181.7 ± 102.1</td>
<td>.000</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>7367.3 ± 1024.8</td>
<td>.000</td>
<td>5373.8 ± 1213.4</td>
<td>.000</td>
<td>1263.3 ± 118.9</td>
<td>.000</td>
<td>1814.6 ± 146.7</td>
<td>.000</td>
<td>1074.6 ± 113.4</td>
<td>.000</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>6915.6 ± 1096.1</td>
<td>.000</td>
<td>4827.0 ± 1257.1</td>
<td>.000</td>
<td>1261.3 ± 120.1</td>
<td>.000</td>
<td>1845.8 ± 118.6</td>
<td>.000</td>
<td>1005.4 ± 102.4</td>
<td>.000</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>6597.8 ± 872.2</td>
<td>.000</td>
<td>4637.7 ± 1466.2</td>
<td>.000</td>
<td>1259.2 ± 193.8</td>
<td>.000</td>
<td>1996.3 ± 183.9</td>
<td>.000</td>
<td>940.4 ± 82.8</td>
<td>.000</td>
</tr>
</tbody>
</table>

*Paired t tests vs pre-drug values.
Noradrenaline Study

There was no change in CCA during the control period (CCA 435.0±79.4 and 434.5±83.4 mL/min, P=NS). The increase in MAP produced by noradrenaline was not significantly different from that produced by L-NMMA at 10 mg/kg (14.5±6.5 versus 18.5±12.0 mm Hg, P=.4). After noradrenaline administration, baseline CCA flow fell from 434.5±83.4 to 379.7±77.1 mL/min, a percentage fall of 12.0±12.1%. This was significantly less than the fall of 37.8±9.9% that followed 10 mg/kg L-NMMA in the hypercapnia study (P<.01). However, there was no significant reduction in the hypercapnic hyperemia response. There was no significant difference in the relative hyperemic responses at either CO2 level between the L-NMMA and noradrenaline groups (Table 2, Fig 3).

Discussion

This is the first published study of which we are aware in which the effect of NOS inhibition on human CBF has been explored. Our results demonstrate that the nonselective NOS inhibitor L-NMMA produces a rapid and dose-dependent fall in basal carotid artery blood flow. This fall was reversed by L-arginine, confirming the specificity of the NOS inhibition. In contrast, there was no significant fall in MCA; in the face of a fall in carotid flow, this suggests that L-NMMA resulted in MCA vasoconstriction. In contrast to results from some studies in animals, we found no evidence that NO contributes significantly to the hyperemia that follows hypercapnia.

The effects on basal CBF are consistent with those found in a large variety of animal studies. L-NMMA also resulted in an increase in MAP, and this may have reduced the magnitude of the fall in CBF seen in response to NOS inhibition. Noradrenaline at an equivalent pressor, and therefore systemic vasoconstrictor, dose to L-NMMA did not reduce CBF to the same degree as L-NMMA, indicating that NO inhibition has a specific effect on the cerebral circulation. In both the L-NMMA and noradrenaline studies, repeated measurements of CBF were made. It has been suggested that CBF may fall with repeated measurements; however, the significantly greater reduction in the L-NMMA group, despite repeated measurements being made in both groups, indicates that the reduction seen after L-NMMA is not due to the effect of repeated measurements but that L-NMMA has a specific effect on basal CBF. Without an independent measure of NOS activity, or a more isoform-selective inhibitor, it is impossible to assess the relative contribution of eNOS and nNOS to our observations.

TABLE 2. Relative Hypercapnic Hyperemic Response Before and After 3 and 10 mg/kg L-NMMA and Before and After Noradrenaline

<table>
<thead>
<tr>
<th>Study</th>
<th>Hypercapnic Responses</th>
<th>6% CO₂</th>
<th></th>
<th>8% CO₂</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute Increase</td>
<td>Increase,</td>
<td>P</td>
<td>Absolute Increase</td>
<td>Increase,</td>
</tr>
<tr>
<td></td>
<td>per kPa</td>
<td>per kPa, %</td>
<td></td>
<td>per kPa</td>
<td>per kPa, %</td>
</tr>
<tr>
<td>L-NMMA hypercapnia Before</td>
<td>46.8±25.0</td>
<td>16.1±6.1</td>
<td>.393</td>
<td>198.0±16.4</td>
<td>57.4±31.7</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>46.4±18.0</td>
<td>.980</td>
<td>19.3±10.2</td>
<td>.240</td>
<td>158.3±94.0</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>42.1±29.1</td>
<td>.754</td>
<td>19.8±15.3</td>
<td>.240</td>
<td>148.0±69.7</td>
</tr>
<tr>
<td>45 min</td>
<td>43.3±10.0</td>
<td>.801</td>
<td>16.4±4.2</td>
<td>.934</td>
<td>138.8±43.4</td>
</tr>
<tr>
<td>Noradrenaline Before</td>
<td>49.1±17.9</td>
<td>12.0±5.7</td>
<td>.384</td>
<td>189.6±60.0</td>
<td>46.4±21.5</td>
</tr>
<tr>
<td>After</td>
<td>55.0±28.0</td>
<td>.627</td>
<td>15.4±8.3</td>
<td>.384</td>
<td>188.6±103.7</td>
</tr>
</tbody>
</table>

For 6% CO₂, results are expressed as both absolute increase and percent increase, both divided by the rise in end-tidal CO₂ in kPa. For 8% CO₂, the absolute and percentage rise, not divided by the rise in CO₂, are given because this level of CO₂ results in maximal vasodilation. Values are mean±SD.
The systemic hypertensive effects of L-NMMA are due to increasing tone in resistance vessels that are normally maintained in a partially vasodilated state by tonic NO release thought to derive from eNOS, and this is supported from studies in eNOS knockout mice. The fall in carotid artery lumen diameter was less so at high CO2 concentrations. However, results in rats have shown conflicting effects of NOS inhibitors on the hypercapnic vasodilatory response, although sometimes with a predominant effect of eNOS. This is supported by the finding that NOS inhibition reduces CBF in wild-type mice but not in eNOS knockout mice.

In contrast to its effects on basal CBF, L-NMMA had no significant effect on the hypercapnic vasodilatory response even at a dose that resulted in significant changes in blood pressure and basal CBF. We estimated this response both as the absolute rise in CBF and the percentage of rise. This lack of inhibition occurred both at moderate concentrations of CO2 (6%) and at higher concentrations (8%) which cause maximal vasodilation. There was a nonsignificant fall in the absolute increase in CCA flow during CO2 administration after L-NMMA, and a nonsignificant rise in the percent increase in CCA flow, but these changes probably reflect the lower baseline flow during L-NMMA administration. Previous animal studies have shown conflicting effects of NOS inhibitors on this response. Most studies in rats have shown NO dependence of the hypercapnic response, although sometimes less so at high CO2 concentrations. However, results in higher species have been less consistent. Inhibition of 20% in cats has been reported, whereas results in primates have shown conflicting results. The reasons for these differences within species are unclear, although newborn animals may show quite different responses from juvenile or adult animals, and there may be differences within different brain regions in NO dependence. Furthermore, in these animal studies, anesthesia may alter cerebrovascular responses. The failure of our study to demonstrate attenuation of the hypercapnic hyperemic response may be a function of the short duration of biological activity of L-NMMA and poor blood-brain barrier penetration in systemic bolus administration. However, blood-brain barrier penetration is not necessary for inhibition of eNOS, and animal studies using a similar route of administration have shown inhibition of the response. One recent study in humans, published in abstract form, suggested that 5 minutes after NOS inhibition with L-NMMA at a dose of 3 mg/kg, CO2 reactivity was reduced. However, because of the effect of L-NMMA on MCA diameter in this situation, velocity does not correlate well with CBF, making the results difficult to interpret.

Human MCA diameter remains constant during a number of physiological alterations, such as blood pressure and CO2 changes. Therefore, MCA diameter is sometimes used as a method of estimating CBF, as in the measurement of cerebral reactivity or autoregulation. While this use is valid in such situations where MCA diameter remains constant, our results suggest that MCA diameter alters with NO inhibition. This is consistent with indirect data suggesting that NO donors dilate the MCA in humans. Therefore, this study provides further evidence that MCA diameter alone cannot be used to study the effect of pharmaceutical agents on CBF if they also affect MCA diameter.

In view of these difficulties in interpreting transcranial Doppler flow velocity changes, we used a quantitative method of measuring CBF, color velocity flow imaging. Doppler-based methods are widely used in measuring carotid volume flow and in assessing the effects of pharmacological agents on the cerebral circulation. Reproducible measurements can be obtained, but inaccuracies can occur because of the need for measurement of the cross-sectional area of the vessel. In color velocity flow imaging, the velocity profile is investigated by a series of small sample volumes. If each velocity component is based measurement of diameter because the calculation relies on the effective diameter of flow whose limit is determined by the minimum velocities detected near the vessel wall. Previous studies using the same equipment as that used in this study have shown that the flow values obtained correlate well with absolute flow values measured by a flowmeter. In humans, the method results in reproducible measurements; the normal values reported in the CCA (330 mL/min in women and 375 mL/min in men) in one study and 376 mL/min in another

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**TABLE 3.** Mean±SD CCA Flow (mL/min) at Rest, 45 min After 10 mg/kg L-NMMA, and After L-Arginine in 4 Subjects Who Received L-Arginine

<table>
<thead>
<tr>
<th></th>
<th>Before L-NMMA</th>
<th>45 Min After 10 mg/kg</th>
<th>After L-Arginine</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>344.0±37.5</td>
<td>268.8±32.4</td>
<td>319.3±27.9</td>
<td>.012</td>
</tr>
<tr>
<td>6% CO2</td>
<td>404.3±42.8</td>
<td>355.3±61.3</td>
<td>421.5±22.2</td>
<td>.15</td>
</tr>
<tr>
<td>8% CO2</td>
<td>470.5±17.9</td>
<td>387.8±54.5</td>
<td>496.0±22.4</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Paired t test after L-arginine vs 45 minutes after 10 mg/kg.
study\textsuperscript{23} are similar to those obtained from xenon radionuclide methods, which estimated CCA flow rates of about 350 mL/min.\textsuperscript{28}

Impaired NO synthesis and release can be demonstrated in the forearm arteries of patients with a number of risk factors for cerebrovascular disease.\textsuperscript{29,30} Now that we have shown that basal CBF in humans is NO dependent, it remains to be determined whether similar impairment of NO release is seen in the cerebral circulation of such patients. In acute stroke, NOS inhibition may have both beneficial and deleterious effects. Animal studies of the effect of NOS inhibition on infarct size have shown conflicting results, but recent studies suggest that nNOS inhibition may be protective while eNOS inhibition may be deleterious, possibly through a fall in CBF.\textsuperscript{31} Our study would support the use of caution with nonspecific NOS inhibitors such as L-NMMA because the potential reduction in tissue perfusion may offset any beneficial neuroprotective effects, although future selective NOS inhibitors may be beneficial.

Acknowledgments

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References

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