Cortical NOS inhibition raises the lower limit of cerebral blood flow-arterial pressure autoregulation

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Cortical NOS inhibition raises the lower limit of cerebral blood flow-arterial pressure autoregulation. Forty-four Sprague-Dawley rats were anesthetized with halothane and O2 to O2. A closed cranial window placed the previous day in a ventilated and physiologically stable preparation, we determined the cerebral blood flow (CBF) using laser-Doppler flowmetry. Animals with low reactivity to inhalation CO2 and suffered ADP or ACH were excluded. Five arterial pressures from 100 to 40 mmHg were obtained with controlled hemorrhagic hypotension under cortical suffusion with artificial cerebrospinal fluid (aCSF) and then again with aCSF, 10−3 M N(Nitro-L-arginine (L-NNA; n = 12), or 10−3 M N(Nitro-b-arginine (o-NNA; n = 7). An additional group (n = 7) was studied after a 105-min suffusion of L-NNA followed by a single blood withdrawal procedure. The lower limit of autoregulation was identified visually by four blinded reviewers as a change in the slope of the five-point plot of CBF vs. mean arterial blood pressure. The lower limit of 90 ± 4.3 mmHg after 105 min of 1 mM L-NNA suffusion was increased compared with the value in the time-control group of 75 ± 5.3 mmHg (P < 0.01; ANOVA) and the initial value of 67 ± 3.7 mmHg (P < 0.001). The lower limit of 84 ± 5.9 mmHg in seven animals with 105 min of suffusion of 1 mM L-NNA without previous blood withdrawal was significantly increased (P < 0.01) in comparison with 70 ± 1.9 mmHg from those with just aCSF suffusion (n = 37). No changes in lower limit for the other agents or conditions, including 105 or 35 min of aCSF or 35 min of L-NNA suffusion, were detected. The lack of effect on the lower limit with o-NNA suffusion suggests an enzymatic mechanism, and the lengthy L-NNA exposure of 105 min, but not 35 min, suggests inhibition of a diffusional distant NOS source that mediates autoregulation. Thus cortical suffusion of L-NNA raises the lower limit of autoregulation, strongly suggesting that nitric oxide is at least one of the vasodilators active during hypotension as arterial pressure is reduced from normal.

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Cerebral blood flow (CBF) pressure autoregulation is the physiological phenomenon that maintains CBF as mean arterial blood pressure (MAPB) varies from about 60 to 160 mmHg (19, 40). As pressure is reduced, CBF flow is maintained by an active vasodilatory process. At the lower limit of autoregulation, a pressure of 60–70 mmHg, this vasodilatory process starts to fail, and blood flow falls linearly in a pressure-passive manner.

Nitric oxide is involved in almost every aspect of cerebrovascular regulation, including reactivity to the partial pressure of carbon dioxide in arterial blood (PaCO2) (22, 56) and functional activation (24, 35). Most investigations of the effect of nitric oxide on CBF regulation use stereospecific inhibitors of nitric oxide synthase (NOS) that are administered intravenously. Because nitric oxide exerts a basal influence on CBF (21), it is likely to be one of the vasodilator substances involved in the seemingly paradoxical vasodilation as flow is decreased. However, studies investigating the role of nitric oxide in CBF-pressure autoregulation have revealed no change in the lower limit or slope of the MAPB-CBF relationship when nitric oxide synthesis was systemically inhibited by intravenous administration of an inhibitor and when local, in contrast to global, CBF was measured (2, 55). Saito et al. (44) investigated the slope of the MAPB-CBF relationship with a local CBF method and pentobarbital sodium anesthesia and showed no effects of NOS inhibition but did not determine the lower limit. Two studies (29, 49) that used local rather than global CBF techniques and CBF measurement at two pressures showed that intravenous NOS inhibition depressed CBF at low pressure compared with higher pressures, supporting the role of nitric oxide in CBF-pressure autoregulation near the lower limit, but did not specifically determine changes in the lower limit. One study (42) in which intravenous NOS inhibition and a local CBF method were used did show an increase in the lower limit, in comparison with both a saline control group and a hypertensive control group. These studies are all complicated by the systemic hypertension produced by intravenous NOS inhibition. In the brain stem, NOS inhibition by suffusion over the brain surface was shown to shift the autoregulatory curve to the right, thus raising the lower limit (51).

To eliminate possible effects from systemic NOS inhibition-induced hypertension, we investigated one aspect of CBF-pressure autoregulation, the lower limit, using NOS inhibition and laser-Doppler flowmetry (LDF) to provide a measure of local cortical flow. We
hypothesize that NOS inhibition via cortical suffusion raises the lower limit of CBF-pressure autoregulation.

**METHODS**

Animal procedures were performed in conformance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the mandated institutional committee concerned with animal procedures. At the end of the experiment, animals were euthanized with a halothane overdose.

Installation of cranial window. To avoid possible trauma to the brain surface, a cranial window was implanted the day before experimental determinations. Forty-four Sprague-Dawley rats [380 ± 7 g (± SE)] were prepared the first day under 1–2% halothane and 70% N2O anesthesia (28–29% O2) administered via endotracheal tube and artificial respiration (model 681, Harvard Apparatus, South Natick, MA). Rectal temperature was maintained at 37°C with a servo-controlled heat lamp (YSI 74, Yellow Springs, OH). After a midline scalp incision and removal of the periosteum, the surface of the skull was flattened to provide a uniform surface for a methyl methacrylate disk (1.5 × 10 mm with a 0.75-mm-thick shelf on one side). An 8-mm-diameter craniotomy was made with a high-speed drill continuously cooled with a blast of nitrogen aimed carefully at the drilling site. The 10-mm-diameter cranial window containing a glued-in thermocouple and three ports, two for artificial cerebrospinal fluid (aCSF) inflow and outflow and one for measuring epicortical pressure, was fixed to the skull with dental acrylic. The animal was given saline (10 ml/kg sc) to prevent postsurgical dehydration and cefazolin sodium (1 mg/kg im; Eli Lilly, Indianapolis, IN) to control possible infection. The scalp was sutured, and the animal was allowed to recover from anesthesia. A suspension of the analgesic acetaminophen (MCIel, Fort Washington, PA) in water (0.5 mg/ml) was presented as drinking water for the animal overnight.

Animal preparation: 2nd day. The next day the animal was anesthetized with 1–2% halothane and 70% N2O in oxygen, a tracheotomy was performed, and artificial ventilation was instituted. Femoral arterial and venous catheters were inserted bilaterally. Halothane was reduced to a maintenance level of ~0.8% by monitoring the blood pressure response to a tail pinch. Gallamine triethiodide (Davis-Geck, Wayne, NJ) in normal saline was infused intravenously at 10 mg·kg⁻¹·h⁻¹. The animal was then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA).

Arterial blood gases [PaCO₂, arterial PO₂ (PaO₂), and pH] and hematocrit concentrations ([Hb]) were determined with a blood gas analyzer (model ABL3, Radiometer America, Westlake, OH). Arterial blood pressure was continuously monitored from a femoral artery, and the epicortical pressure from the cranial window port was monitored with strain-gauge transducers (model DT-XX, Viggo Spectramed, Oxford, CA). The aCSF solution (37) was bubbled with 6% CO₂-10% O₂-84% N₂ at 37°C and pumped at 0.5 ml/min to the cranial window. The aCSF was prepared in a special atmosphere with 6% CO₂-10% O₂-84% N₂ at 37°C and pumped at 0.5 ml/min to the cranial window. The aCSF was withdrawn (PaCO₂pre). After end-tidal CO₂ indicated that a plateau had been reached, and if a brisk CBF response occurred, a second blood gas sample (PaCO₂post) was drawn. If the CO₂ reactivity was absent, the probe was repositioned to another cortical area, and the CO₂ reactivity was determined again. Once the CO₂ reactivity was acceptable, the response to infused gases was studied by the equations CO₂ reactivity = (CBFpre)/(CBFpre)/(PaCO₂pre)/(PaCO₂post) - PaCO₂adp. ADP or ACh reactivity = (100 × (CBFpost-CBFpre))/(CBFpre)/(PaCO₂post - PaCO₂pre) and ADP or ACh reactivity = (100 × (CBFpost - CBFpre)/(CBFpre)). If CO₂ reactivity was <1% mmHg⁻¹ or if the ADP or ACh reactivity was <10% or 15%, respectively, the animal was not used.

Hemorrhagic hypotension. The protocol for five of the six groups (Table 1) included two incidents of hemorrhagic hypotension, separated by a suspension period of 35 or 105 min. Blood was withdrawn from the arterial femoral catheter into a heparinized 10-ml syringe until a mean arterial pressure of 100 mmHg was reached. As shown in Fig. 1A, this pressure was maintained for 3–4 min by careful adjustment of the volume of blood in the syringe. This procedure was repeated for arterial pressures of 85, 70, 55, and 40 mmHg over a 20-min period. The mean total volume of withdrawn blood was 4.1 ± 0.3 ml. Respirator adjustments, deduced in pilot experiments, were made during hemorrhagic hypotension to minimize the drop in PaCO₂. For the double blood withdrawal...
protocol, blood was slowly reinfused. Suffusion was either continued with aCSF or changed to either 10^{-3} M N^\text{\textsubscript{\textgamma}}-nitro-L-arginine (L-NNA) or 10^{-3} M N^\text{\textsubscript{\textgamma}}-nitro-D-arginine (D-NNA) for 35 or 105 min, followed by another sequence of controlled hemorrhagic hypotension.

MABP was recorded just before blood was withdrawn to reach the initial target pressure of 100 mmHg. Blood gases and MABP were determined at the beginning (target MABP 100 mmHg) and end (target MABP 40 mmHg) of each blood withdrawal.

Experimental groups and measurements. Five groups were studied with the double blood withdrawal protocol, providing two repeated determinations of the lower limit in the same animal (Table 1). The control value of the lower limit was determined after 90 min of aCSF suffusion. A 105-min interval of either aCSF (CTRL-105 group; \( n = 10 \)), L-NNA (L-NNA-105 group; \( n = 12 \)), or D-NNA (D-NNA-105 group; \( n = 5 \)) suffusion or a 35-min interval of aCSF (CTRL-35 group; \( n = 5 \)) or L-NNA (group L-NNA-35; \( n = 5 \)) suffusion was then followed by a second blood withdrawal and lower limit determination. In an additional group (L-NNAS-105; \( n = 7 \)), a 105-min suffusion with L-NNA was followed by a single blood withdrawal procedure. The purpose of this single blood withdrawal group was to ensure that the reinfusion of blood in the double blood withdrawal group L-NNA-105 had no effect on the subsequent lower limit.

As a gauge of the level of vessel constriction after reinfusion, the CBF at a blood pressure of 100 mmHg at the beginning of the first blood withdrawal (CBF\textsubscript{1st WD}) was compared with the CBF at a blood pressure of 100 mmHg at the beginning of the second blood withdrawal (CBF\textsubscript{2nd WD}) as CBF\textsubscript{2/1} = 100 × (CBF\textsubscript{2nd WD})/(CBF\textsubscript{1st WD}). For the single blood withdrawal protocol (group L-NNAS-105), a control CBF measurement at a blood pressure of 100 mmHg before the L-NNA suffusion was compared with the CBF at the beginning of the blood withdrawal sequence at a blood pressure of 100 mmHg.

Determination of lower limit. As shown in Fig. 1, A and B, for each blood withdrawal sequence, five pairs of measurements of average MABP and CBF were taken over 128-s periods of stable MABP obtained just before the next pressure drop. The CBF (in arbitrary units) at an arterial pressure of 100 mmHg was used as the control (100%) CBF value as shown in Fig. 1C. A separate autoregulatory curve (see Fig. 1D) for each animal and blood withdrawal, constructed from these five pairs of MABP vs. CBF (as percentage of control), was plotted with a code number but without animal or group identification. Four blinded reviewers were instructed to identify the lower limit of autoregulation as the pressure at which the plateau in CBF starts to fall. There were two other types of autoregulatory curves in addition to the classic plateau followed by a fall (27) that required specific instruc-

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Reinfusion</th>
<th>Suffusion Agent</th>
<th>Before 1st WD</th>
<th>Before 2nd WD</th>
<th>Suffusion Time, min</th>
</tr>
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<tbody>
<tr>
<td>CTRL-105</td>
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<td>aCSF</td>
<td>aCSF</td>
<td>aCSF</td>
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<td>L-NNA-105</td>
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<td>105</td>
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<td>D-NNA</td>
<td>105</td>
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<td>L-NNAS-105</td>
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<td>aCSF</td>
<td>L-NNA</td>
<td>L-NNA</td>
<td>105</td>
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<tr>
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<td>aCSF</td>
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<td>35</td>
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<tr>
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<td>aCSF</td>
<td>L-NNA</td>
<td>L-NNA</td>
<td>35</td>
</tr>
</tbody>
</table>

Group names and suffusion protocols are described in detail in Experimental groups and measurements; \( n = \) no. of animals/group. CTRL, control; 1st WD and 2nd WD, first and second blood withdrawals. Suffusion agents: aCSF, artificial cerebrospinal fluid; L-NNA, 10^{-3} M N^\text{\textsubscript{\textgamma}}-nitro-L-arginine; D-NNA, 10^{-3} M N^\text{\textsubscript{\textgamma}}-nitro-D-arginine. There was no "1st WD" in single-withdrawal L-NNA (L-NNAS)-105 group, but aCSF was suffused. Mean lower limit of 37 1st WD animals was used for comparison with single "2nd WD" of L-NNA-105 group.

As shown in Fig. 1, A and B, for each blood withdrawal sequence, five pairs of measurements of average MABP and CBF were taken over 128-s periods of stable MABP obtained just before the next pressure drop. The CBF (in arbitrary units) at an arterial pressure of 100 mmHg was used as the control (100%) CBF value as shown in Fig. 1C. A separate autoregulatory curve (see Fig. 1D) for each animal and blood withdrawal, constructed from these five pairs of MABP vs. CBF (as percentage of control), was plotted with a code number but without animal or group identification. Four blinded reviewers were instructed to identify the lower limit of autoregulation as the pressure at which the plateau in CBF starts to fall. There were two other types of autoregulatory curves in addition to the classic plateau followed by a fall (27) that required specific instruc-

Fig. 1. Experimental record of mean arterial blood pressure (MABP) and cerebral blood flow (CBF) from laser-Doppler flowmetry (LDF). Traces of MABP (A) and CBF (B) from an experimental record show blood withdrawal sequence. MABP decreased from 100 to 40 mmHg in 5 15-mmHg steps over 16 min. CBF is expressed in arbitrary units (au) from LDF probe placed on surface of cranial window. Bars above each step are 128-s epochs over which average MABP and CBF data pairs were obtained. C start and stop times for each epoch, mean CBF, mean MABP, and CBF (%). D: autoregulatory curve, plotted from CBF (%control) and MABP data pairs in C, was presented to 4 blinded reviewers to estimate lower limit of autoregulation. Mean of their estimates at 69 mmHg for this curve.

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differences in MABP were intentional. Because all PaO2 analysis.
been raised to at least this pressure (28). The average of the pressure passive, we assumed that the lower limit was at
was excluded. If the second blood withdrawal curve was still
defined by a continuous fall in CBF with at least a 15%
decrease by 70 mmHg, we attempted to use a CBF-MABP
data pair at a higher pressure immediately previous to the first 100-mmHg point, if one existed. If none existed, the animal was excluded. If a data pair from a higher pressure did exist, a six-point plot was produced, and the curve was then regraded in an attempt to define a lower limit. If the first blood withdrawal curve was still pressure passive, we assumed that the lower limit was at least 100 mmHg, on the assumption that the lower limit had been raised to at least this pressure (28). The average of the lower limits from the four reviewers was used for further analysis.

Chemicals. ADP, ACh, and L-NNA were obtained from Sigma Chemical (St. Louis, MO). D-NNA was obtained from Bachem California (Torrance, CA).

Statistics. The consistency and agreement among the reviewers in identifying the lower limit was tested using the intraclass correlation coefficient (ICC). The ICC is estimated using variance components from ANOVA and can be interpreted as a correlation coefficient. Repeated-measures ANOVA was performed to test for differences between physiological variables, the lower limit of autoregulation, and values of CBF. Linear combinations of treatment means were used to test differences among groups. Paired or unpaired t-tests were used for all comparisons. Values are expressed as means ± SE. Significance was assumed when P < 0.05. The statistical analyses were performed with the SAS system (45); n values indicate number of animals.

RESULTS

Physiological variables. There were no differences among groups in MABP, pH, or PaCO2. However, there were significant differences among groups for PaO2 and [Hb] (P < 0.05). Table 2 presents the physiological variables over time. There were differences over time for MABP, PaCO2, PaO2, pH, and [Hb] (P < 0.001). The differences in MABP were intentional. Because all PaO2 values were between 110 and 155 mmHg, O2 saturation was always >95%. Mean [Hb] ranged from 18.9 to 14.2 g/dl, but these differences were not of sufficient magnitude to affect CBF. Both PaCO2 and [Hb] dropped slightly from the values at a MABP of 100 mmHg to the values at 40 mmHg. For instance, mean PaCO2 for the 1st blood withdrawal was 36 mmHg at 100 mmHg and 33 mmHg at the lower pressure, but these differences are of little significance to measurements of CBF in relation to normal PaCO2 reactivity of 2.5% mmHg⁻¹ (26). These differences in physiological variables were not sufficient to influence CBF or the lower limit of autoregulation, were controlled for by the repeated-measures experimental design with the time-control groups CTRL-105 and CTRL-35, and were of a similar pattern in each group.

Reviewer consistency. The four reviewers evaluated 81 curves (37 animals had 2 curves and 7 had 1 curve) using supplied instructions. The ICC was 0.83 (P < 0.001; <2% of the total variance in the lower limit data can be attributed to the reviewer. This high level of agreement provides evidence that the instructions for estimating the lower limit were clear, easily understood, and produced consistency.

Lower limit of autoregulation. The lower limits of autoregulation in the six groups are presented in Fig. 2. In the L-NNA-105 group, the lower limit was significantly increased to 90 ± 4.3 mmHg after 105 min of L-NNA suffusion in comparison with 1) the time control, the lower limit after 105 min of aCSF suffusion in the CTRL-105 group (75 ± 5.3 mmHg; P < 0.01, ANOVA; n = 10); 2) the initial value of 67 ± 3.7 mmHg determined during the first blood withdrawal under aCSF (P = 0.001; n = 12); and 3) the lower limit from the first blood withdrawal mean of 71 ± 2.2 mmHg (P = 0.001; n = 25) for the other four groups. In addition, the lower limit from the animals in group L-NNA-105 (84 ± 6 mmHg; n = 7) subjected to only one blood withdrawal after 105 min of L-NNA suffusion was significantly increased in comparison with the lower limit of 70 ± 1.9 mmHg (P = 0.01; n = 37) from the grouped aCSF 1st blood withdrawal animals from all the double blood withdrawal protocols. The lower limits after L-NNA suffusion in the L-NNA-105 group (84 ± 6 mmHg) and L-NNA-105 (90 ± 4.3 mmHg) groups were similar. Thus suffusion for 105 min with 1 mM L-NNA raised the lower limit in relation to the time-control group (2nd blood withdrawal, L-NNA-105 vs. CTRL-105), over time in the L-NNA-105 group (1st vs. 2nd blood withdrawal), and without blood reinfusion (L-NNA-105 vs. all other 1st blood withdrawal lower limits).

There was no change in the lower limit after blood reinfusion and 105 min of aCSF (group CTRL-105: 66 ±

Table 2. Physiological variables

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MABP, mmHg</th>
<th>PaCO2, mmHg</th>
<th>PaO2, mmHg</th>
<th>pH</th>
<th>[Hb], g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44</td>
<td>108 ± 1</td>
<td>36.2 ± 0.3</td>
<td>127 ± 3</td>
<td>7.472 ± 0.006</td>
<td>18.1 ± 0.2</td>
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<tr>
<td>Pre-1st WD</td>
<td>37</td>
<td>105 ± 1</td>
<td>32.8 ± 0.5</td>
<td>140 ± 3</td>
<td>7.472 ± 0.007</td>
<td>14.8 ± 0.4</td>
</tr>
<tr>
<td>Post-1st WD</td>
<td>37</td>
<td>40 ± 0.3</td>
<td>35.5 ± 0.3</td>
<td>123 ± 3</td>
<td>7.447 ± 0.009</td>
<td>17.2 ± 0.2</td>
</tr>
<tr>
<td>Control pre-2nd WD</td>
<td>44</td>
<td>111 ± 2</td>
<td>33.1 ± 0.4</td>
<td>136 ± 3</td>
<td>7.450 ± 0.006</td>
<td>14.6 ± 0.2</td>
</tr>
<tr>
<td>Pre-2nd WD</td>
<td>44</td>
<td>105 ± 1</td>
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</table>

Values are means ± SE; n = no. of animals. For 1st WD values, n = 37 because the 7 animals in L-NNA-105 group are considered to have had a "sham" 1st WD. Their single WD occurred at sametemporal position as 2nd WD of other animals. Only MABP is available for control and control pre-2nd WD. For statistical analysis, see text. Pre-WD values are at a target MABP of 100 mmHg; post-WD values are at a target MABP of 40 mmHg. PaCO2 and PaO2, arterial PCO2 and PO2, respectively; [Hb], hemoglobin concentration.
The major finding of this study is that local NOS inhibition in the cerebral cortex raises the lower limit of autoregulation in cortex as shown schematically in Fig. 2. Lower limits of autoregulation after cortical suffusion with various agents. Lower limit of autoregulation (means ± SE) for 1st and 2nd blood withdrawals (1st WD and 2nd WD) for 5 double-WD groups [CTRL-105, N^\text{-}nitro-L-arginine (L-NNA)-105, N^\text{-}nitro-D-arginine (D-NNA)-105, CTRL-35, and L-NNA-35] and for 1st WD for single-WD group (L-NNA_2-105) are shown. For description of groups, see Table 1 and Experimental groups and measurements. Lower limit for double-WD protocol (L-NNA-105 group) after 105 min of L-NNA suffusion was significantly increased to 90 ± 4.3 mmHg compared with control lower limit of 67 ± 3.7 mmHg (P < 0.001; n = 12) and in comparison with second lower limit after 105 min of artificial cerebrospinal fluid (aCSF) suffusion for CTRL-105 group (75 ± 5.3 mmHg; P < 0.01, n = 10). Lower limit for single-WD protocol with 105 min of L-NNA suffusion (L-NNA_2-105 group; 84 ± 5.9 mmHg) was significantly increased (P = 0.01; n = 7) from 1st WD mean lower limit under aCSF from all double-WD protocols (70 ± 1.9 mmHg; n = 37). No change in lower limit was noted in any other experimental manipulation. **P < 0.01; ***P < 0.001.

3.7 to 75 ± 5.3 mmHg), indicating that both time and blood reinfusion did not change the lower limit. Similarly, suffusion with D-NNA, the enantiomer of L-NNA, did not change the lower limit (group D-NNA-105: 73 ± 5.5 to 82 ± 6.5 mmHg), suggesting a stereospecific enzymatic role for the effect of L-NNA. Suffusion for a shorter interval of 35 min with aCSF or 1 mM L-NNA also did not change the lower limit in groups CTRL-35 or L-NNA-35 (76 ± 3.2 to 80 ± 5.8 or 72 ± 4.8 to 75 ± 6.6 mmHg, respectively), suggesting a diffusionally distant source for the effect of L-NNA suffusion for 105 min.

LDF. The changes in CBF from the beginning of the first blood withdrawal to the beginning of the second withdrawal, calculated as CBF_2, for the six groups are presented in Fig. 3. Increases in CBF to 159 ± 22% of control group (P < 0.001 from control) occurred in the time control CTRL-105 group and in the enantiomer control D-NNA-105 group to 152 ± 24% (P = 0.03 from control). After 105 min of L-NNA suffusion, CBF values in both the L-NNA-105 (95 ± 7%) and L-NNA_2-105 (131 ± 29%) groups were not different from control CBF values. For both 35-min suffusion groups, the CBF values were not different from control values.

DISCUSSION

The major finding of this study is that local NOS inhibition in the cerebral cortex raises the lower limit of autoregulation in cortex as shown schematically in Fig. 2.

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was shown to shift the autoregulatory curve to the right, thus raising the lower limit.

Speculative mechanisms for nitric oxide as the vasodilator near the lower limit. Proposed mechanisms of vasodilation (and constriction) during autoregulation deal mostly with either pressure-sensitive mechanisms involving intrinsic properties of smooth muscle (known as the myogenic response) or flow-mediated mechanisms that typically involve release of autacoids from endothelium (known as the metabolic hypothesis) (40). Most likely, both the myogenic and the flow-mediated systems are operative and could be additive during vasodilation induced by hypotension (31).

Much evidence exists that nitric oxide is released from endothelium as shear stress increases, both from in vivo studies (48), from studies that used excised arteries (11, 17), and from in vitro studies that used cultured endothelial cells exposed to fluid flow (43). Flow-sensitive vasodilation involving shear stress on endothelium has received little attention as a mechanism applied to the vasodilation during induced hypotension that is characteristic of autoregulation because a decrease in pressure and flow velocity in a rigid tube would lead to a decrease in shear stress, not an increase. Our results appear to be in contrast to this because they suggest that nitric oxide is released as pressure drops and shear stress decreases, which is seemingly at odds with the evidence that nitric oxide is released as shear stress increases. However, careful consideration of the mechanical forces in the vessel wall after a drop in arterial pressure might rectify this discrepancy. K Kontos et al. (30) observed in cats that pial arteriolar diameter decreased by 6% in 3 s during the initial decrease in pressure from about 100 to 40 mmHg, presumably due to the passive contraction of elastic elements in the vascular wall. Thus if we assume that flow velocity remained constant during this initial 3 s in the work by K Kontos et al. (30), this 6% collapse would cause a 20% increase in shear stress at the vessel wall because shear stress changes are related to vessel diameter by the inverse cube (17). Such a transient increase in shear stress during induced hypotension could presumably cause release of nitric oxide from endothelium (17). This mechanism is entirely speculative, with regard to both the work by Kontos et al. (30) and the results of this work. The actual mechanism for the nitric oxide-mediated vasodilation suggested by our results has yet to be elucidated. Other possibilities include a mechanism whereby a decrease in shear stress-mediated nitric oxide release could influence the release of a secondary vasodilator, and by removing this decrease in nitric oxide release this secondary vasodilator would also be diminished.

Source of nitric oxide. Possible locations of the NOS that is inhibited by cortical \( L^-NNA \) suffusion include perivascular nerves, astrocytes, neurons, and endothelium (21). An endothelial location of NOS is supported by the recent report that CBF is depressed at arterial pressures near the lower limit in endothelial NOS (eNOS) knockout compared with wild-type mice (20). Although it is impossible to infer this location from our data, the ineffectiveness of the shorter suffusion of 35 min of NOS inhibitor compared with the 105-min suffusion period suggests that the inhibited NOS was diffusively distant. Our data indicate that 35 min of suffusion is not long enough to permit \( L^-NNA \) to inhibit the NOS that is the source of nitric oxide that mediates the vasodilation near the lower limit, but that 105 min of \( L^-NNA \) exposure does allow inhibition of this NOS source. Diffusional barriers to suffused agents have been reported in the pial circulation (7) and are probably even more important for intraparenchymal arteries such as penetrator arterioles. The lack of change in the lower limit with 35 min of suffusion of \( L^-NNA \) is consistent with the lack of effect until 60 min of \( L^-NNA \) suffusion in some parameters used to assess functional activation (24) and CBF and pial artery diameter response to \( CO_2 \) (23), although changes in other parameters were noticeable after shorter periods of \( L^-NNA \) suffusion. In contrast, Fabricius et al. (9) showed rapid decreases in NOS activity and \( CO_2 \) reactivity within 5–15 and 30–45 min, respectively, after cortical \( L^-NNA \) suffusion. However, the timing of the effect of NOS inhibition on the \( CO_2 \) reactivity, cGMP, or functional activation, or even NOS activity assayed by the ex vivo NOS assay, might be different from the timing for its influence on the lower limit. Different and multiple anatomic and cellular locations of either or both of the constitutive isoforms of NOS contribute to this issue.

Consider that the NOS in the tissue volume sensed by the laser-Doppler probe must be fully inhibited before a change in CBF can be detected. If our laser-Doppler probe illuminates to a median depth of 1 mm, \( L^-NNA \) must diffuse and inhibit NOS to at least this depth. Our data showing that 105 min of \( L^-NNA \) suffusion did, and 35 min did not, affect the lower limit are consistent with autoradiographic observations using \( ^4C^-L^-NNA \) showing that 1 mM \( L^-NNA \) suffused for 2 h gives a concentration of 0.25 mM, and for 30 min gives a concentration of 0.10 mM, at a depth of 1 mm (12).

Influence of exclusion of animals with lessened vascular reactivity on the lower limit. ADP vasodilation has both endothelium-dependent and -independent components, ACh reactivity is endothelium dependent and related to eNOS, and \( CO_2 \) reactivity is related to neuronal NOS (22, 56). If we suspect that inhibition of eNOS, or neuronal NOS, is responsible for the increase in the lower limit, then it is possible that exclusion of an animal because of a lessened reactivity to ACh or \( CO_2 \) would influence the results. This possibility prompted an analysis of the relation between ACh and \( CO_2 \) reactivity and the lower limit. With the use of ACh or \( CO_2 \) reactivity as the independent variable and the lower limit as the dependent variable, linear regression showed no significant correlation for ACh or \( CO_2 \) reactivity \( (r^2 = 0.27 \ (P > 0.10) \) and \( r^2 = 0.0095 \ (P > 0.5) \), respectively), suggesting that the exclusion of subjects with low ACh reactivity or \( CO_2 \) reactivity would not bias the evaluation of the lower limit.

Double blood withdrawal design. The advantage of the double blood withdrawal design in studies of auto-
regulation is that it permits statistical analysis with the more sensitive repeated-measures methods. This design is not often used because of concern about the effect of reinfusion of the withdrawn blood on cerebrovascular responses. In this study, the lower limit in the L-NNA-105 group with a single blood withdrawal after 105 min of L-NNA suffusion was similar to that in a double withdrawal group (L-NNA-105), indicating that the reinfusion of blood did not affect the increase in lower limit from L-NNA suffusion. The reinfusion of previously withdrawn blood did increase the CBF for the start of the second blood withdrawal in the aCSF time-control group (CTRL-105) but did not change the lower limit in comparison with the first blood withdrawal measurement. The difference between the lower limit after 105 min of L-NNA suffusion in the L-NNA-105 group and 105 min of aCSF suffusion in the CTRL-105 group was evident despite the slight nonsignificant increase in the lower limit in the CTRL-105 group, presumably caused by hyperemia or some other common factor related to reinfusion. Thus the strength of the repeated-measures ANOVA design was demonstrated in this protocol, and the experimental design deals appropriately with the reinfusion effect by providing comparison with time-control groups.

CBF after L-NNA suffusion. In contrast to the changes in the lower limit of autoregulation caused by NOS inhibition, cortical suffusion for 105 min with 1 mM L-NNA did not change CBF in either the L-NNA-105 or L-NNA-105 groups. NOS inhibition is usually associated with slight decreases in cortical flow: flow decreased to 71% with topical 1 mM L-NNA (6), although no changes in pial arterial diameter (41) or CBF (8, 9) were noted after topical NOS inhibition for 45 min. In groups CTRL-105 and D-NNA-105 after a second aCSF or D-NNA suffusion, there was an increase in CBF to 159 ± 22% and 152 ± 24%, respectively, of control animals, most probably attributable to the reinfusion of blood after the first blood withdrawal. Fitch et al. (10) observed an almost equivalent rise of ~166% in CBF after reinfusion under similar conditions in the baboon. Perhaps this hyperemia after reinfusion is mediated by nitric oxide via an unknown mechanism and was abolished by NOS inhibition, and the lack of flow depression in the L-NNA-105 group without reinfusion is a characteristic of this preparation and corresponds to the results of others who did not observe a depression of flow (8, 9) or pial arterial diameter (41) with cortical NOS inhibition.

NOS inhibition induced vasoconstriction. Even though we used cortical suffusion to produce local NOS inhibition to eliminate the systemic hypertensive effects, we were initially concerned that the local vasoconstrictive effect of NOS inhibition, which usually causes a reduction in CBF, would affect the lower limit. Although the direct assessment of vessel constriction is not possible with measurements of CBF, relative changes in constriction can be inferred by comparison of CBF before the 1st blood withdrawal and CBF before the 2nd withdrawal because both measurements are at the identical MABP of 100 mmHg. In groups L-NNA-105 and L-NNA-105, as shown in Fig. 3, no change of CBF was observed due to L-NNA suffusion, whereas in groups CTRL-105 and D-NNA-105, increases in CBF were observed, presumably due to blood reinfusion. We maintain that the observed increases in the lower limit were independent of these changes in CBF because 1) in groups CTRL-105 and D-NNA-105, the increased CBF after reinfusion at the equivalent MABP of 100 mmHg would suggest vasodilation and an increased lower limit, whereas we observed no change in the lower limit in these groups; and 2) in groups L-NNA-105 and L-NNA-105, there was no change in CBF at equivalent MABP, suggesting equivalent tone, but we observed increases in the lower limit. The increased CBF, presumably mediated by sympathetic activation after blood reinfusion (14), could have been balanced by the decrease in flow due to NOS inhibition in the L-NNA-105 and L-NNA-105 groups but not in the CTRL-105 and D-NNA-105 control groups. Thus our initial concern that the constrictive effect of NOS inhibition would modify the lower limit was not substantiated because the observed changes in the lower limit were different from those predicted on the basis of this concern.

The lower limit in rats. Although the use of reviewers to assess the lower limit of autoregulation has been rare, our determination agrees with the results obtained by many workers. As summarized in Table 3, our lower limit of 70 ± 1.9 mmHg under control conditions that used blinded reviewers and individual autoregulatory curves agrees with those of others who used a variety of methods for CBF determination, hypotension induction, and assessment of the lower limit. It is interesting to note that the lower limit was much lower in those studies that used barbiturates [47 mmHg (42) and 45 mmHg (51)] than in the studies that used a variety of other anesthetics (70 mmHg is the mean of all other observations presented in Table 3). This observation is in concert with the depressive effect of the barbiturates on most cerebral circulatory parameters.

Intravenous NOS inhibition and local vs. global lower limits. Several studies that reported no involvement of the NOS system in CBF-pressure autoregulation utilized intravenous NOS inhibition and global (2, 55) or local (44) CBF measurements. Global assessments of CBF combine brain regions that react differently to arterial pressure decreases and are known to have different lower limits (36, 49). It is well accepted that the deeper, more archaic cytoarchitectural structures are protected against ischemia and hypotension (3); these structures possess (49) a lower limit of CBF-pressure autoregulation that is less than that of the telencephalon. The cerebral venous outflow method used by Buchanan and Phillis (2) includes blood derived from deep structures as well as the cortex and complicates the assessment of changes in the lower limit. It is also possible that in these other brain regions, a vasodilator different from nitric oxide could be mediating vasodilation near the lower limit. In another study that used dogs under pentobarbital
Table 3. The lower limit of autoregulation in rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Anesthesia</th>
<th>Method of Hypotension</th>
<th>Method of CBF</th>
<th>Method of Lower Limit</th>
<th>Lower Limit, mmHg</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>Ketamine</td>
<td>HH</td>
<td>$H_2$ clearance</td>
<td>MABP &gt; CBF, $\leq 60$</td>
<td>60</td>
<td>Van Wylen et al. (52)</td>
</tr>
<tr>
<td>WKY</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>MABP &gt; CBF, $\leq 80$</td>
<td>80</td>
<td>Barry et al. (1)</td>
</tr>
<tr>
<td>Wistar</td>
<td>Awake</td>
<td>Recovery from hemorrhage</td>
<td>$H_2$ clearance</td>
<td>MABP &gt; CBF, $\leq 90$</td>
<td>90</td>
<td>Lartaud et al. (33)</td>
</tr>
<tr>
<td>SD</td>
<td>Halothane</td>
<td>Reservoir hypotension</td>
<td>LDF</td>
<td>MABP &gt; CBF, $\leq 60$</td>
<td>60</td>
<td>Verhaegen et al. (53)</td>
</tr>
<tr>
<td>Wistar</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 76$</td>
<td>76</td>
<td>Wang et al. (55)</td>
</tr>
<tr>
<td>SD</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>LDF</td>
<td>MABP &gt; CBF, $\leq 60$</td>
<td>60</td>
<td>Lee et al. (34)</td>
</tr>
<tr>
<td>SD</td>
<td>Ketamine</td>
<td>HH</td>
<td>LDF</td>
<td>MABP &gt; CBF, $\leq 60$</td>
<td>60</td>
<td>Morita et al. (39)</td>
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<tr>
<td>SD</td>
<td>Halothane</td>
<td>[Intracranial pressure]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 73$</td>
<td>73</td>
<td>Hauerberg and Uhler (15)</td>
</tr>
<tr>
<td>SD</td>
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<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 89$</td>
<td>89</td>
<td>Hauerberg et al. (16)</td>
</tr>
<tr>
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<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 68$</td>
<td>68</td>
<td>Larsen et al. (32)</td>
</tr>
<tr>
<td>WKY</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 60$</td>
<td>60</td>
<td>Vraamark et al. (54)</td>
</tr>
<tr>
<td>WKY</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Schmidt, $\leq 77$</td>
<td>77</td>
<td>Torup et al. (50)</td>
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<tr>
<td>Wistar</td>
<td>Pentobarbital</td>
<td>Gradual HH</td>
<td>LDF</td>
<td>Schmidt, $\leq 43$</td>
<td>43</td>
<td>Preckel et al. (42)</td>
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<tr>
<td>Wistar</td>
<td>Chloral hydrate</td>
<td>LBNP</td>
<td>LDF</td>
<td>MABP &gt; CBF, $\leq 50$</td>
<td>50</td>
<td>Heimann et al. (18)</td>
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<tr>
<td>SD</td>
<td>Amobarbital</td>
<td>HH</td>
<td>LDF</td>
<td>MABP &gt; CBF, $\leq 45$</td>
<td>45</td>
<td>Toyota et al. (51)</td>
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<tr>
<td>SD</td>
<td>Halothane</td>
<td>[MABP and HH]</td>
<td>$^{133}$Xe (ic)</td>
<td>Visual inspection, $\leq 70$</td>
<td>70*</td>
<td>Gross et al. (13)</td>
</tr>
<tr>
<td>SD</td>
<td>Halothane</td>
<td>HH</td>
<td>LDF</td>
<td>Blinded reviewer, $\leq 70$</td>
<td>70*</td>
<td>This study</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto; SD, Sprague-Dawley; HH, hemorrhagic hypotension; [MABP and HH], increased MABP with a pharmacological agent before induction of HH; LBNP, lower body negative pressure; $^{133}$Xe (ic), intracarotid $^{133}$Xe injection; LDF, laser-Doppler flowmetry; Schmidt, multiple linear regression method for determining lower limit (46); MABP > CBF, lower limit is taken as MABP just higher than pressure at which cerebral blood flow (CBF) is significantly lower than control. *Mean of lower limit excluding measurements with barbiturate anesthesia.

sodium anesthesia (44) with CBF determinations at multiple MABPs, NOS inhibition had no effect on the autoregulatory curves or cerebrovascular resistance, but if the data are plotted, it appears that the lower limit is $<40$ mmHg. This depressed lower limit is similar to those of other studies that used barbiturates in rats (42, 51) (Table 3). Thus if Saito et al. (44) had used another anesthetic or evaluated lower MABPs, changes in autoregulation from NOS inhibition might have been observed.

Intravenous NOS administration causes systemic vasoconstriction by blocking tonic release of nitric oxide from endothelium, leading to moderate hypertension. When intravenous NOS inhibition is used to evaluate autoregulation, a rise in arterial pressure occurs that does not occur in the control group. Comparison of a normotensive control group with a hypertensive NOS-inhibited group is a problematic experimental design for assessment of the lower limit. The hypertensive effect of intravenous NOS inhibition may be controlled by one of two strategies: 1) the control group may be made hypertensive, pharmacologically, to the same degree as the NOS-inhibited group; or 2) the NOS-inhibited group may be made normotensive, using hemorrhage or a pharmacological agent. Neither of these strategies is ideal. Either one of these strategies produces equivalent starting MABP in the control and treated groups, so the lower limit can be compared from an equivalent starting point. Our restriction of NOS inhibition to the brain avoids these potential complications.

Autoregulation studies can be difficult to interpret unless a CBF-MABP curve is obtained using multiple data pairs, because changes in the lower limit cannot be distinguished from changes in the slope of the plateau. Two studies that used autoradiographic (49) and transillumination (29) CBF methods showed changes in autoregulation with systemic NOS inhibition with only two CBF-MABP data pairs. These changes can be ascribed either to a change in the slope of the autoregulatory plateau or to a change in the lower limit. In contrast, another study (2) that used two CBF-MABP data pairs showed no effect of NOS inhibition on autoregulation.

Implications for cerebral ischemia. The importance of the lower limit of autoregulation for the circulatory events involved in the pathogenesis of cerebral ischemia is well accepted. Our results that suggest a role for nitric oxide as a vasodilator near the lower limit of autoregulation are consistent with the evidence that nitric oxide is beneficial in the initial acute phase of cerebral ischemia, presumably because it acts as a vasodilator in the ischemic penumbra (4). Thus the early therapeutic administration of either a nitric oxide donor or a precursor such as L-arginine (38) could be beneficial. Alternatively, if the lower limit can be raised by pharmacological manipulations that involve NOS inhibition, then our data suggest that caution may be warranted if NOS inhibitors were to become useful therapeutic agents for some other diseases, especially in situations where cerebral ischemia may be imminent or suspected or when acute hypotension is a possibility.

In conclusion, these results implicate nitric oxide as a vasodilator that modulates CBF autoregulation in the range of MABP between 60 and 90 mmHg (Fig. 4), although other vasodilatory mechanisms in the pressure range of 60–100 mmHg are by no means excluded. At higher pressures, a vasodilator different from nitric oxide or a vasoconstrictor could participate in the autoregulatory process.

Intact and normal vasodilatory mechanisms were documented in these studies. A delay of 1 day after surgery was imposed to avoid the disruption of cerebral circulatory regulatory mechanisms from surgical
trauma. In addition, central features of the experimental design were: 1) the individual autoregulation responses were analyzed rather than averaged; 2) the double and single blood withdrawal protocols gave virtually identical increases in the lower limit after L-NNA suffusion, indicating that sequential hemorrhagic hypotension with a repeated-measures design is an effective method of study for lower-limit determination, despite reinfusion hyperemia; and 3) different initial levels of flow do not necessarily affect the lower limit of the autoregulatory response.

Thus L-NNA suffusion raises the lower limit of autoregulation. Although our finding is in contrast to those of most previous workers, these data suggest that either the effects of systemically administered NOS inhibitors somehow mask their capacity to alter the lower limit of autoregulation or local measurements of CBF provide a more sensitive measure of autoregulation, possibly due to known regional differences in autoregulation.

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