Nitric Oxide Promotes Arteriolar Dilation During Cortical Spreading Depression in Rabbits

David M. Colonna, MD; Wei Meng, MD; Dwight D. Deal, BS; David W. Busija, PhD

Background and Purpose: Pial arterioles transiently dilate during cortical spreading depression (CSD), although the mechanisms are unclear. We tested the hypothesis that increased production of nitric oxide (NO) promotes arteriolar dilation.

Methods: Urethane-anesthetized rabbits were equipped with cranial windows, and the diameter (reported in micrometers) of a pial arteriole was determined via intravital microscopy. In each rabbit, a baseline CSD was elicited by micropipette application of KCl onto the cortex, and resultant pial arteriolar dilation was monitored. Either 100 μmol/L Nω-nitro-L-arginine methyl ester (L-NAME) or 50 μmol/L Nω-nitro-L-arginine methyl ester (L-NA), both competitive NO synthase inhibitors, was then applied to the brain surface. A CSD was elicited as before. The L-NAME and L-NA were then removed by artificial cerebrospinal fluid washes. An additional CSD was induced with KCl as before.

Results: Control CSD in the L-NAME group dilated pial arterioles: baseline diameter, 66±7 mm, with CSD=106±8 mm (59% increase). After topically applied L-NAME, CSD dilated pial arterioles less; baseline diameter, 61±7 mm, with CSD=77±6 mm (26% increase), P<.05 compared with control CSD diameter. Topical L-NA had similar effects on CSD: control CSD dilated pial arterioles 51%; after topical L-NA, only 14% (P<.05). After removal of L-NAME or L-NA, CSD-induced pial arteriolar dilation was similar to original control values.

Conclusions: The reversible inhibition of CSD-induced pial arteriolar dilation by either L-NAME or L-NA suggests that NO contributes to arteriolar dilation observed with CSD. (Stroke. 1994;25:2463-2470.)

Key Words: • nitric oxide • spreading cortical depression • rabbits

Cortical spreading depression (CSD), a wave of depolarization followed by repolarization of cortical neurons,1,2 is accompanied by transient depression of neuronal electrical activity, dilation of pial arterioles,3+ and elevation of cerebral blood flow (CBF).7 CSD may play a role in the aura phase of migraine vascular headache in humans,4 although recent evidence suggests that CSD is not the initiating event in migraine attacks.9 Also, CSD occurs spontaneously during other pathological brain conditions, such as focal ischemic insults10 and traumatic brain injury.11 The mechanisms responsible for cerebrovascular dilation during CSD are complex and not fully understood. It is likely that during CSD, parenchymal and pial arterioles are exposed to different vasoactive influences. For example, superfusion of the cortical surface with artificial cerebrospinal fluid (aCSF) during CSD does not change the magnitude or duration of pial arteriolar dilation in urethane-anesthetized rabbits,4 indicating that these responses stem from the release of factors intrinsic to the arteriolar wall. These intrinsic substances may act directly on pial arterioles, activate perivascular nerves, or cause the release of vasodilator substances from the vascular endothelium. However, parenchymal arterioles are likely exposed to elevations of extracellular potassium ([K+]c),12 adenosine, neurotransmitters, and prostaglandins.

Recent studies have shown that nitric oxide (NO), or a related nitrosothiol,12,13 is an endogenous vasodilator substance and, although its role remains controversial, it is a logical candidate as a promotor of pial arteriolar dilation during CSD, for two reasons. First, activation of N-methyl-D-aspartate (NMDA) receptors occurs during CSD,14 and NMDA stimulates the release of NO from brain cells.15 Second, in recent CSD experiments on cats reported by Goadsby et al.,16 in which local cortical blood flow was measured by laser Doppler, intravenous L-NAME, a putative NO synthase (NOS) inhibitor,17 ablated the hyperemic response normally observed with CSD for more than 2 hours. This evidence suggests that in the cat model, the CSD hyperemic response appears mediated to a large degree by NO. On the other hand, two recent reports...
suggest that NO release cannot be responsible for observed cerebral hyperemia during CSD.\textsuperscript{18,19} In these two studies, a rat model of CSD was used with L-NAME and L-NA, respectively, as NOS inhibitors. Potential limitations of these studies were the reliance on L-NAME or L-NA as the sole NOS inhibitor, the failure to test the ability of cerebral resistance vessels to dilate on exposure to non-NO-mediated dilative stimuli, and the lack of direct evidence that NO activity was actually inhibited by L-NAME or L-NA. In contrast to other NOS inhibitors, such as L-NAME, L-NAME possesses muscarinic receptor antagonist properties in some tissues.\textsuperscript{20} Further, in the study by Goadsby et al.,\textsuperscript{16} responses of surface arterioles, which probably are not influenced directly by parenchymal metabolites,\textsuperscript{2} were not examined specifically.

Because of the incomplete and conflicting nature of the evidence evaluating the role of NO in CSD-induced hyperemia,\textsuperscript{16,18,19} we tested the hypothesis that topically applied NOS inhibitors, L-NAME and L-NA, would attenuate pial arteriolar dilation during CSD in the rabbit. Further, we examined the ability of L-NAME to decrease cortical NO activity and to selectively inhibit NO-dependent dilation of cerebral arterioles. Our experiments validated our hypothesis that NO promotes pial arteriolar dilation during CSD in the rabbit.

Methods

Animal Preparation

Female New Zealand White rabbits were used in these experiments and were housed, fed, and handled according to NIH/USDA standards, and protocols were approved by the Animal Care and Use Committee of Wake Forest University. For the following series of experiments, the rabbits were initially anesthetized with 10 to 15 mg/kg of thiopental, administered via a marginal ear vein. Urethane was administered at 1 g/kg IP, with 0.5 g/kg in subsequent doses if needed. Additional urethane and thiopental were immediately given if signs of light anesthesia were present: spontaneous movement, aversive movements to noxious stimuli, or changes in blood pressure or respiratory rate suggesting a light anesthetic plane. All surgical sites were infiltrated with 1% lidocaine before incision. A catheter (PE-90, Clay Adams) was inserted to measure body temperature, which was maintained at 38°C with a heating blanket. A tracheostomy was then performed. The rabbit's head was secured in a stereotactic frame. A craniectomy 1.8 cm in diameter was performed through the parieto-occipital skull. The cortex was exposed by going through the dura mater. A cranial window composed of a steel ring of the same diameter as the craniectomy with a glass coverslip affixed was secured in the craniectomy with dental acrylic. This ring was machined with three injection ports for the administration of drugs to the craniectomy. One hole was the site for insertion of a short length of PE-50 tubing as a guide for the microapplication of 10 \(\mu\)L of 5% KCl to initiate CSD. A Ag-AgCl electrode (World Precision Instruments, Inc) was placed into the second burr hole to demonstrate the slow DC potential change that is characteristic of CSD,\textsuperscript{3,4} the other criterion for demonstrating the presence of CSD. Both the EEG tracings and the DC slow potential tracing were continuously recorded on the Grass polygraph.

The window was then flushed with aCSF to purge air bubbles that may have been trapped under the glass coverslip. The aCSF was formulated to match that of a rabbit, heated to 38°C, and gassed with a 6.5% CO\(_2\)/66% O\(_2\)/balance N\(_2\) mixture before injection into the cranial window. The composition of the aCSF was as follows (in mmol/L): KCl 2.9, MgCl\(_2\) 0.6, CaCl\(_2\) 1.3, Na\(_2\)HPO\(_4\) 13.19, urea 6.7, dextrose 3.7, and NaHCO\(_3\) 20.2.

The image of arterioles was projected onto a color television screen by use of a microscope (Wild, model M3B) and video camera (Panasonic Digital 5100). The diameter of the vessel was continuously recorded with a video scaling device (model IV-550, FOR-A Corp, Ltd) with output to the polygraph.

A number of arginine analogues have been shown to inhibit the activity of NO. In the following experiments, we used topically applied L-NAME (Sigma) as a NOS inhibitor.\textsuperscript{17} Since L-NAME also possesses muscarinic receptor antagonist properties, we performed additional experiments using L-NA (Sigma), a NOS inhibitor\textsuperscript{17,21,22} without apparent muscarinic receptor activity.\textsuperscript{20} All solutions of L-NAME and L-NA were freshly made in aCSF, warmed to 38°C, and gassed with O\(_2\)/N\(_2\)/CO\(_2\) before administration.

Experimental Protocols

Validation of Topical L-NAME as an Effective Inhibitor of Acetylcholine-Induced Dilation

When acetylcholine (ACH) is applied to blood vessels having an intact endothelium, there is a dose-dependent dilation produced by the release of an endothelium-dependent relaxing factor, which is NO or an NO-containing compound.\textsuperscript{12,13,23} We applied to the brain surface 10-\(\mu\)mol/L ACh and 100-\(\mu\)mol/L ACh (n=5 animals) solutions freshly prepared in aCSF and observed pial arteriolar response. We then topically applied a 100-\(\mu\)mol/L solution of L-NAME mixed in aCSF, waited 15 minutes, and sequentially administered the same doses of ACh as described above with 100 \(\mu\)mol/L L-NAME.

L-NAME-, L-NA-, and Adenosine-Induced Pial Arteriolar Dilation

This series of experiments was performed in 6 rabbits to assess whether L-NAME and L-NA possess the ability to inhibit pial arteriolar dilation in this animal model via some nonspecific, non-NOS-mediated mechanism. Adenosine is a known, potent dilator of rabbit cerebral arterioles\textsuperscript{24} and appears to dilate vessels through several non-NOS-mediated mechanisms: (1) adenosine produces an increase in intracellular cyclic AMP;\textsuperscript{25} (2) adenosine decreases inward Ca\(_{\text{2+}}\) movement;\textsuperscript{26} and (3) adenosine may activate the sarcolemmal Na\textsuperscript{+}-K\textsuperscript{+} pump in cerebrovascular smooth muscle.\textsuperscript{27} In this series of experiments, a pial arteriole was identified and the diameter measured throughout the experimental period. Freshly prepared solutions were made of adenosine (Calbiochem) in aCSF. Ten and 100 \(\mu\)mol/L adenosine were applied onto the brain (others have demonstrated that 100 \(\mu\)mol/L produced near-maximal pial arteriolar dilation in the rabbit),\textsuperscript{28} and the diameter was measured. The animals were then randomized to receive either topical 100 \(\mu\)mol/L,
L-NAME or 50 μmol/L L-NA, and the response to adenosine was rechecked as outlined below in Table 1.

### Topical L-NAME and NO Synthase Activity

We used an assay of NOS activity modified from that described by Colonna et al.26 We measured basal NOS activity in rabbit brain cortex and after topical application of 100 μmol/L L-NAME. NO synthase converts [14C]arginine into equimolar amounts of NO and [14C]citrulline. We rapidly harvested brain cortex from control (n=4) and L-NAME–administered (n=4) animals and flash-froze the samples in liquid nitrogen. At the time of assay, the samples were placed in 2 mL ice-cold buffer (50 mmol/L Tris and 2 mmol/L EDTA, pH 7.4) and ultrasonicated for 10 seconds. The samples were centrifuged at 10,400g for 15 minutes at 4°C, and the supernatant (cytosol and membranes) was collected. For each assay, 25 μL of supernatant was added to 100 μL of reaction mixture containing 1 mmol/L NADPH, 1.25 mmol/L CaCl₂, and 1 μmol/L [14C]arginine. Incubation was carried out at 37°C (3 control and 2 L-NAME animals) and 22°C (1 control and 2 L-NAME animals) for 30 minutes. The reaction was terminated with 2 mL iced buffer: 30 mmol/L HEPES, 3 mmol/L NaCl, 0.5 mmol/L EDTA, pH 5.5. Each sample was placed in a chromatography column of 0.5 mL Dowex AG50W–X8 (Na+ form) and eluted with 2 mL deionized water. [14C]Citrulline was quantified by liquid scintillation spectroscopy, with an efficiency of 90%. Protein concentration of each sample was quantified by the method of Bradford.27 Because two different reaction temperatures were used on the different days the assays were performed, thereby affecting enzyme kinetics, the results are expressed as percent inhibition of NOS activity by L-NAME compared with control.

### Topical L-NAME and CSD

For these CSD experiments, 9 rabbits were used. A pial arteriole was identified and the diameter followed throughout the experimental time period. We did not measure the vascular responses of pial veins during CSD, since minimal changes in surface vein diameter occur during CSD.4 All CSDs were induced by the microapplication of 10 μL of 5% KCl, and at least 15 minutes elapsed between CSDs to allow for stabilization of the observed arteriole. A baseline CSD was elicited. Two separate washes of 100 μmol/L L-NAME were introduced onto the brain surface, and a second CSD was elicited. After the L-NAME was washed out with ACSF and after at least 15 minutes had passed, a postwash CSD was elicited. In several animals, more than one ACSF wash and CSD were required to remove the NOS antagonist sufficiently to elicit a dilative response with CSD similar to that observed during baseline responses.

### Topical L-NA and CSD

These experiments in 6 rabbits were performed in exactly the same fashion as those discussed above except that 50 μmol/L L-NA was topically applied instead of L-NAME. This dose was chosen upon review of recent work by Faraci and Breese,28 in which a similar dose of topical L-NA effectively inhibited pial arteriolar dilation to 1 and 10 μmol/L ACh in an identical rabbit cranial window preparation. These experiments were performed to assess whether L-NA, an arginine analogue that does not demonstrate muscarinic receptor antagonism, would attenuate arteriolar dilation during CSD.

### Statistical Analysis

Values are reported as mean±SEM. Comparisons between two values were made with simple t tests or a one-way ANOVA; for comparisons within subjects, repeated-measures (RM) ANOVA and paired t tests were used. If the F value was significant after an RM ANOVA, pairwise comparisons were made by use of the Student-Newman-Keuls test. The α level was set at .05 for all statistical tests. The α levels for testing comparison significance were adjusted for multiple comparisons by Bonferroni’s method. For the data presented in Tables 1 and 2, the change in arteriolar diameter for each arteriole during CSD was calculated (CSD diameter minus pre-CSD diameter) for each of the three treatments (baseline, L-NAME or L-NA, and ACSF wash). An ANOVA was performed to test for differences in arteriolar diameter between the three treatments. Factors included in the statistical model were treatment, pre-CSD MAP, and change in MAP (CSD MAP minus pre-CSD MAP). Contrasts were performed to compare treatment pairs. Percent change of arteriolar diameter during CSD (Figs 3 and 4) was expressed as the mean±SEM of the percent changes, preserving the paired structure of the data.

### Results

#### ACh Responses With and Without L-NAME

The two doses of topical ACh used in these experiments, 10 and 100 μmol/L, proved to be potent dilators of pial arterioles. ACh yielded a 46% and 60% increase in arteriolar diameter, respectively. Coadministration of ACh in the same concentrations as before with 100 μmol/L L-NAME markedly reduced the dilative response of ACh, P<.05 (Fig 1).

#### L-NAME, L-NA, and Adenosine-Induced Pial Arteriolar Dilation

Six animals were used in these experiments. Ten and 100 μmol/L adenosine dilated pial arterioles 18±4% and 39±5%, respectively (Fig 2). These amounts of dilation with these two concentrations of adenosine are comparable to those reported by other researchers.21 Application of either 100 μmol/L L-NAME or 50 μmol/L L-NA failed to attenuate the dilation observed by either dose of adenosine (Fig 2). These data suggest that application of L-NAME or L-NA at these concentrations does not, through some nonspecific mechanism, impair the ability of pial arterioles to dilate.

### Topical L-NAME and NO Synthase Activity

In the 8 animals for which NOS activity was assayed (4 control and 4 topical L-NAME animals), there was a 46±14% decrease in cortical NOS activity.
CSD With and Without L-NAME

The 9 animals used in this series of experiments were hemodynamically stable throughout the study period (Table 2), with no significant differences between the MAP values immediately before or during the CSDs. In Fig 3, percent dilations during CSD in the baseline and aCSF wash groups compare favorably to that measured in previous studies.\(^3\) Of import, Fig 3 illustrates the significant \((P<.05)\) diminution of CSD-induced dilation when L-NAME was topically applied before CSD (L-NAME group).

L-NA and CSD

In these 6 animals, the CSDs measured in the absence of L-NA (Fig 4) were similar to those in the above-described L-NAME experiments. L-NA application significantly diminished pial arteriolar dilation during CSD compared with responses in the absence of L-NA \((P<.05)\). These animals were hemodynamically stable before, during, and after CSD (Table 3).

Discussion

Our results demonstrate that topical administration of the NOS inhibitors L-NAME and L-NA reversibly inhibits pial arteriolar dilation during CSD in anesthetized rabbits. Also, administration of these agents in identical doses failed to attenuate arteriolar dilation.
after application of a known cerebral vasodilator, adenosine, suggesting that topical administration of these NOS inhibitors did not produce nonspecific inhibition of arteriolar dilation. Further, the dose of L-NAME used inhibited NOS activity in rabbit cortical tissue by 46%. The fact that NOS blockade by L-NAME was demonstrated by our NOS assay and the fact that arteriolar dilation from 100 μmol/L topical ACh (a stimulant for NO synthesis) reduced by 75% with this dose of L-NAME strongly suggest that NOS activity was diminished after L-NAME administration. Since application of L-NA before CSD inhibited arteriolar dilation comparably to that observed with L-NAME and since L-NA does not possess any known antimuscarinic properties, it is unlikely that the effect of L-NAME on CSD is mediated through any putative antimuscarinic properties. In addition, Faraci and Breese found that topical L-NA (100 μmol/L) in a similar rabbit cranial window preparation inhibited NO production after application of NMDA and ACh. Thus, the diminution of pial arteriolar dilation during CSD after application of either NOS inhibitor, L-NAME or L-NA, suggests that NO is a promotor of this vascular response. In light of the evidence presented here, however, it is difficult to determine precisely what portion of the arteriolar response during CSD is attributable to NO, for two reasons. First, there are CGRP-, substance P (SP)-, and neurokinin A3,32 peptide-containing perivascular nerves that invest pial arterioles. These peptides are all vasodilators and probably contribute to pial arteriolar dilation during CSD to a greater or lesser degree. Second, the dose of L-NAME chosen in these experiments inhibited cortical NOS by 47%. Our assay protocol was not capable of discerning to what degree NOS was inhibited in cortical neurons, vascular endothelium, or perivascular NOS-containing (nitrooxidergic) neurons. Although we feel confident that NO promotes pial arteriolar dilation during CSD, we do not know what fraction of the overall response is directly attributable to NO.

The potential sources of NO production and release during CSD are open to speculation but were not specifically tested with these experiments. During CSD, pial arteriolar vascular endothelium could be stimulated to produce NO via at least four different pathways. First, NO could be released from cerebral vascular endothelium or perivascular NOS-containing (nitrooxidergic) neurons. Although we feel confident that NO promotes pial arteriolar dilation during CSD, we do not know what fraction of the overall response is directly attributable to NO.

Table 2. Pial Arteriolar Diameter and Mean Arterial Pressure Measurements During CSD in the Presence and Absence of L-NAME

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<th>Baseline</th>
<th>L-NAME</th>
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<td></td>
<td>Pre-CSD</td>
<td>During CSD</td>
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<tr>
<td>Diameter, μm</td>
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<td></td>
<td>42±8</td>
<td>(53±11)</td>
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<td>Change (Mean %)</td>
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<td>MAP, mm Hg</td>
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CSD indicates cortical spreading depression; L-NAME, Nω-nitro-L-arginine methyl ester; aCSF, artificial cerebrospinal fluid; and MAP, mean arterial pressure. There were no statistical differences between the MAP values over time (P = .539, repeated-measures [RM] ANOVA). *Significant decrease in arteriolar diameter during CSD in the presence of L-NAME present compared with baseline and aCSF washout response (P < .05, RM ANOVA). †Significant decrease in mean arteriolar diameter change and ± mean percent increase in vessel diameter during CSD with L-NAME present compared with baseline and aCSF washout response (P < .05, RM ANOVA). Values are mean ± SEM.

Table 3. Pial Arteriolar Diameter and Mean Arterial Pressure Measurements During CSD in the Presence and Absence of L-NA

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CSD indicates cortical spreading depression; L-NA, 50 μmol/L Nω-nitro-L-arginine; aCSF, artificial cerebrospinal fluid; and MAP, mean arterial pressure. There were no statistical differences between MAP values over time (P = .880, repeated-measures [RM] ANOVA).
Pial arteriolar diameter was measured via intravital microscopy. Topical application of 100 μmol/L L-NA significantly reduced pial arteriolar dilation during CSD, suggesting NO to be a promotor of CSD-induced cerebral hyperemia.

In a unique model of CSD in awake rats, Duckrow revealed that L-NAME (30 mg/kg IV) administration revealed a brief period of cortical hypoperfusion, a 64% decrease in CBF, at the onset of CSD (we did not observe pre-CSD arteriolar constriction). In the baseline condition without L-NAME present, this initial hypoperfusion was not present. After L-NAME administration, the magnitude of cortical hyperemia normally observed during CSD was decreased from a baseline 45% increase in flow to only 38%, not a significant change. The author concluded that NO does not mediate cerebral hyperperfusion in rats. Supporting this finding, another study showed that the administration of L-NA to anesthetized rats failed to alleviate cerebral hyperemia during CSD. Indeed, these authors found that intravenous or topical application of N^N-nitro-L-arginine actually enhanced the hyperemia induced by CSD. Once again, these investigators failed to demonstrate effectiveness or specificity of NOS blockade.

A recent publication by Buxton et al suggests that L-NAME possesses muscarinic receptor antagonist properties. The role, if any, of muscarinic receptor activation during CSD is unclear. Our experimental design addresses this issue by including an additional NOS inhibitor, L-NA, in the experimental design. L-NA, which does not possess any apparent muscarinic receptor antagonist potential, demonstrated similar efficacy in inhibiting pial arteriolar dilation during CSD compared with responses with L-NAME. Thus, our results are consistent with the view that L-NAME in our preparation produces NOS blockade independent of any significant antimuscarinic effects.

It is likely that other substances in addition to NO may be acting as mediators coupling CBF to the increase in brain metabolism that is observed in the acute phase of CSD. Experiments in this laboratory revealed that CGRP is released during CSD, stimulating dilation of pial arterioles. Additional substances may act as mediators coupling CBF to increased metabolism during CSD. For example, Shibata et al discovered that prostaglandins in CSF increase markedly during and after CSD in rats; however, the prostaglandins liberated were primarily vasoconstrictive in nature, promoting pial constriction during CSD. Thus, treating the rabbits with intravenous indomethacin increased pial arteriolar dilation and the hyperemia (measured by laser Doppler) observed during CSD.

One aspect of our experimental model deserves comment: we chose to make arterial blood gas measurements during the course of the experimental time period. Typically, blood gas measurements were made just after the cranial window was inserted and at two more time points afterward. We did not measure blood gases during the period of CSD itself. Thus, if CSD induced a change in our experimental subjects' respiratory patterns sufficient to produce hypoventilation, then hypoxemia and/or hypercapnia could produce dilation of pial arterioles. We do not believe this to be a plausible explanation of our observations, for several reasons. First, we never observed apnea during CSD. Second, other investigators have used an identical
model of CSD except for mechanical ventilation of the rabbits. Compared with our results, they reported similar degrees of pial arteriolar dilation ipsilateral to the CSD, whereas contralateral arterioles did not dilate. Third, hypoxemia or hypercapnia would produce dilation of pial veins. Although we did not measure pial vein diameter in our experimental design, no gross dilation of pial veins was observed during CSD that would have been present had anpca occurred.

In summary, two inhibitors of NOS, L-NAME and L-NA, attenuated pial arteriolar responses to CSD, whereas responses to topical adenosine remained intact. Thus, NO or a related nitrosothiol appears to contribute to CSD arteriolar dilation in the rabbit. Corroborating evidence in cat models provides strong evidence that NO promotes cerebrovascular dilation during CSD. However, species differences may still be important in this response, since two reports of rat CSD models have failed to demonstrate that NO mediates cerebral hypereemia during CSD. The source or sources of NO liberated during CSD are unclear and require further study.

Acknowledgments

This study was supported by grants HL-30260 and HL-46558 from the National Institutes of Health, National Heart, Lung, and Blood Institute, Forsyth County United Way, and NIH grant RR-05404. The authors extend thanks to Wilson Somerville for his generous help in editing this manuscript, to Adela Larimore for transcribing the manuscript, and to Robert James for his help in the statistical analysis of data. Special thanks are offered to Dr Joseph R. Tobin for his help in developing our NOS assay.

References

Colonna et al demonstrate the ability of inhibitors of nitric oxide synthase (NOS) to significantly reduce pial arteriolar dilation accompanying cortical spreading depression (CSD) in rabbits studied using a cranial window preparation. $N^\omega$-nitro-L-arginine methyl ester (L-NAME) and $N^\rho$-nitro-L-arginine (L-NA) markedly blunted the pial arteriolar response to CSD, and L-NAME inhibited cortical NOS activity by 47%; these findings strongly suggest a large contribution of cortical nitric oxide (NO) or related nitrosothiol to the vascular hyperemia associated with CSD. In general this is a nicely done study, and convincingly implicates NO as one of the mediators responsible for the pial arteriolar vasodilation associated with CSD. However, NO- and CSD-associated hyperemia appears to be species-dependent in that it has been observed in rabbits and cats but is resistant to NOS blockade in rats. Thus, insofar as CSD is a model of enhanced metabolic activity, these findings suggest that NO may be one of the mediators linking neuronal activity to flow. Species variations, however, make it difficult to suggest that there is such a relationship in humans. Furthermore, data from the present study do not allow one to determine the cell type responsible for NO production during CSD, nor to determine whether the release of NO is a primary event associated with neuronal activation and depolarization or whether NO is released secondarily through the action of metabolites or neurotransmitters.

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*Stroke*. 1994;25:2463-2470
doi: 10.1161/01.STR.25.12.2463

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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