0031-3998/93/3403-0354$03.00/0
PEDIATRIC RESEARCH

Vol. 34, No. 3, 1993
Printed in U.S.A.

Effects of N-ω-Nitro-L-Arginine Methyl Ester on the Cerebral Circulation of Newborn Piglets Quantified *In Vivo* by Near-Infrared Spectroscopy

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ABSTRACT. The effects of N-ω-nitro-L-arginine methyl ester (L-NAME) on basal cerebral vascular tone, the vasodilatory effects of acetylcholine (ACh), and the cerebrovascular response to alterations in arterial carbon dioxide tension (CBVR) were investigated using near-infrared spectroscopy. Seven newborn piglets were anesthetized and mechanically ventilated; mean arterial blood pressure (MAP) was monitored and near-infrared spectroscopy used to measure changes in total cerebral Hb concentration. At the beginning of the experiment, CBVR was measured and then 10, 20, 30, and 100 mg·kg^{-1} L-NAME were administered sequentially; ACh (1, 2, 3, and 5 μg) was given before and after each injection of L-NAME. At the end of this sequence, CBVR was measured again and finally sodium nitroprusside (1.5 mg·kg^{-1}) was administered. Ten and 20 mg·kg^{-1} L-NAME caused a significant decrease in total cerebral Hb concentration of −0.59 (−3.21 to −0.02) and −1.46 (−6.50 to −0.15) μmol·L^{-1} (median and range), respectively (Wilcoxon $p < 0.05$), but subsequent injections did not. Ten, 20, and 100 mg·kg^{-1} L-NAME caused an increase in MAP (Wilcoxon $p < 0.05$). ACh caused an increase in total cerebral Hb concentration and a decrease in MAP that was impaired but not abolished by L-NAME (ANOVA $p < 0.05$). CBVR was not affected by L-NAME. Sodium nitroprusside caused a reduction in mean (SD) MAP of 4.7 (1.6) kPa, and a slower rise in [tHb] of 13.44 (2.03) μmol·L^{-1}. Postmortem examination of three animals revealed NADPH-diaphorase staining in neurons, cerebral blood vessels, carotid artery, and jugular vein. These results demonstrate that nitric oxide modulates basal vasodilation and part of the vasodilatory action of ACh in the newborn piglet brain, but that these effects are quantitatively small. Nitric oxide was not shown to be an important participant in CBVR. This is consistent with the suggestion that the mechanism mediated by nitric oxide may coordinate perfusion rather than control bulk flow in the developing brain. (*Pediatr Res* 34: 354–359, 1993)

Abbreviations

ACh, acetylcholine
CBVR, response of [tHb] to changes in $PaCO_2$
L-NAME, N-ω-nitro-L-arginine methyl ester
MAP, mean arterial blood pressure
NIRS, near-infrared spectroscopy
NO, nitric oxide
$PaCO_2$, arterial carbon dioxide tension
PaO_2, arterial oxygen tension
[tHb], total cerebral Hb concentration

Received August 31, 1992; accepted April 15, 1993.
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Supported by the Medical Research Council, the Wellcome Trust, the Wolfson Foundation, and Hamamatsu Photonics KK.

NO is synthesized from L-arginine and oxygen in a variety of cells, including endothelia, neurons, platelets, and macrophages. There are at least two forms of NO synthase. One is constitutive and responds to physical or neurotransmitter signals with a brief burst of NO synthesis, increasing cGMP and regulating cell function or communication. Another form of enzyme is inducible, and NO released over a prolonged time may have a role in cytotoxicity. NO derived from endothelium has been shown to modulate tone in many vascular beds (1). However, there are comparatively few data available on its role in the cerebral circulation in the perinatal period, especially from measurements made *in vivo* (2, 3). We have used the noninvasive technique of NIRS to obtain quantitative data *in vivo* on the role of NO in the cerebral vascular control of the newborn piglet to test the hypothesis that NO is a mediator of cerebrovascular control.

L-NAME, a specific inhibitor of NO synthesis, was used to examine the basal secretion of NO, the release of NO stimulated by ACh, and the participation of NO in the CBVR. Sequential doses of ACh were used to define the dose-related vascular response to stimulation of NO secretion; the administration of increasing doses of L-NAME allowed the effect of inhibition of NO synthesis on this response to be determined. The changes in [tHb] and MAP that occurred with each dose of L-NAME were recorded to demonstrate alterations in baseline vascular tone resulting from inhibition of basal NO secretion. Before and after this sequence, CBVR was measured, and at the end of the experiment sodium nitroprusside was given to test the response of the cerebral vasculature to exogenous NO. Postmortem examination of the piglet brain, carotid artery, and jugular vein was also undertaken to detect the presence of NADPH-dependent NO synthase.

MATERIALS AND METHODS

NIRS. This technique depends on measuring the characteristic absorption of near-infrared light by the chromophores oxyhemoglobin and deoxyhemoglobin within cerebral tissue (4). Details of the NIRS system developed at this institution have been

described elsewhere (5). For this study a commercial prototype spectrophotometer (NIR 1000, Hamamatsu Photonics KK, Hamamatso City, Japan) was used. Briefly, near-infrared light at six wavelengths between 779.0 nm and 907.8 nm was carried to the piglet's head through a fiberoptic bundle, the end of which (the optode) was applied to the parietal region. Light emerging from the head was collected by another optode placed over the contralateral parietal area and transmitted to the photomultiplier tube of the spectrometer by a second fiberoptic bundle. The distance between the optodes, always greater than 3.5 cm, was measured with mechanical callipers. To prevent interference from background illumination, the animal's head was wrapped in a light-excluding bandage.

Changes in the intracerebral concentrations of oxy- and deoxyhemoglobin were calculated from the modified Lambert-Beer law by the least squares curve-fitting technique using previously established extinction coefficients (6) and assuming an optical pathlength of 4.39 times the distance between the optodes (7). Measurements were made continuously, averaged into variable time bins for display and recorded on computer disk for later analysis. The sum of oxy- and deoxyhemoglobin is equal to the [tHb], which is directly related to the cerebral blood volume by the cerebral hematocrit.

Animal preparation. Seven piglets, body weight 1.3–1.7 kg, were studied at less than 24 h of age. Anesthesia was induced using 5% halothane and maintained with i.v. fentanyl (5 $\mu g \cdot kg^{-1}$) and pentobarbitone (20 $mg \cdot kg^{-1}$), repeated as necessary. The piglets were wrapped in manually controlled warming coils and intubated with a 3.5 or 4.0 mm endotracheal tube (Portex, Hythe, UK). Mechanical ventilation was provided by a pressure-limited ventilator (Vickers Medical, Sidcup, UK) at 30 breaths/min, with an inspiratory time of 0.5 s, a peak inspiratory pressure of 1.0–1.2 kPa and an inspired gas mixture of oxygen and room air, adjusted as necessary to maintain normal $PaCO_2$ and PaO_2. The rectal temperature and ECG were monitored continuously (HP 78201B, Hewlett-Packard Co, Palo Alto, CA), and intermittent determinations made of arterial blood gases (Radiometer, Copenhagen, Denmark) and glucose (BMstix, Boehringer Mannheim, Germany). Catheters (3.5 F) were inserted through the umbilical artery and vein: a continuous infusion ($80 mL \cdot kg^{-1} \cdot d^{-1}$) of 0.15% saline and 4% glucose was given through the venous catheter, which was also used for drug administration, and the arterial catheter was connected to a strain gauge transducer system (Elcomatic, Glasgow, UK) for continuous blood pressure monitoring and withdrawal of blood samples.

CBVR. A sample of arterial blood was taken for blood gas measurement immediately before an alteration in ventilatory rate that induced a change in $PaCO_2$ of a median of 1.2 (range 0.9 to 1.6) kPa. An additional blood gas estimation was then made and the procedure repeated. The inspired oxygen concentration was varied to keep the PaO_2 as constant as possible. CBVR was calculated as the ratio between changes in [tHb] and changes in $PaCO_2$ (8).

Experimental protocol. A period of stabilization lasting at least 80 min was allowed after preparation; during this time $PaCO_2$ was maintained between 4.8 and 5.9 kPa and halothane was not administered. CBVR was then estimated. Next, four bolus injections of ACh (Sigma, Poole, UK) were given to stimulate NO release (1, 2, 3, and 5 μg) and the relation between the dose and its effects on [tHb] and MAP determined. NO synthesis was then inhibited by sequential bolus injections of L-NAME (Sigma) at doses of 10, 20, 30, and 100 $mg \cdot kg^{-1}$. (In one piglet, only one injection of 10 $mg \cdot kg^{-1}$ was given.) After each injection of L-NAME, administration of ACh (1, 2, 3, and 5 μg) was repeated. The mean (SD) interval between each injection of L-NAME was 28.5 (7.6) min. After a cumulative dose of 160 $mg \cdot kg^{-1}$ of L-NAME, CBVR was measured again, a mean (SD) of 34.3 (11.9) min after the final dose of ACh. Finally, 1.5 $mg \cdot kg^{-1}$ of sodium nitroprusside (Sigma) was given. The vehicle for drug injections was 0.9% saline, and this was also used for control injections (n = 16), which were isovolumetric with drug administrations. At the end of the experiments, the piglets were killed by an overdose of the anesthetic urethane.

Postmortem examinations. Immediately after death, the brains, carotid arteries, and jugular veins of three piglets were removed and placed in Hanks' physiologic salt solution. The carotid arteries and veins were opened and stretched out onto Sylgard silicon rubber (Dow Corning, Seneffe, Belgium) and fixed with 4% paraformaldehyde for 2 h at 4°C. They were then washed three times in PBS containing 0.1% Triton X-100 for 10 min each in preparation for staining for NADPH-diaphorase. The brain was cut into small pieces and fixed with 4% paraformaldehyde at 4°C overnight. The pieces were washed several times in 7% sucrose solution and left overnight in the same solution at 4°C. The brain tissues were then frozen onto cork in tissuetek (Miles Scientific, Naterville, IL) embedding medium by immersion in precooled isopentane followed by liquid nitrogen. Ten-μm thick sections were cut using a cryostat and transferred to gelatin rubbed slides for staining. NADPH-diaphorase staining was performed by incubating the tissues with 1.2 mM β-NADPH, 0.24 mM nitroblue tetrazolium, 15.2 mM L-malic acid, and 0.5% Triton X-100 in 0.1 M Tris-HCl (pH 7.6) for 2 h at 37°C. Incubation times were between 30 and 45 min. After incubation, tissues were rinsed in PBS, mounted in Citifluor (City University, London) mountant and viewed with bright field illumination.

Data analysis and statistical methods. To measure the changes in [tHb] and MAP due to L-NAME, ACh, sodium nitroprusside, or 0.9% saline, values from periods of at least 1 min immediately before injection were averaged to provide reference values. After injections, the period of maximum change was averaged; after L-NAME, sodium nitroprusside, and 0.9% saline, a 2-min period was taken, but after ACh, a 30-s period was defined because of the transient nature of the response. After all injections except sodium nitroprusside, the period of maximum change occurred within 6 min of injection.

Data groups were assessed by inspection of distributions, means, and medians and using the K-S test. When the distributions were approximately Gaussian, parametric tests were applied; otherwise nonparametric statistics were used. When analysis of variance was used, residuals were examined for deviation from normality and the Cochrane-C method was used to test for equality of variance. The changes in [tHb] and MAP associated with the injection of L-NAME were compared with the alterations induced by 0.9% saline injections using the Wilcoxon rank sum test. The change in [tHb] and MAP due to injection of ACh and the effect of the consecutive doses of L-NAME on this change were examined by analysis of variance. CBVR before and after L-NAME was compared by the Wilcoxon rank sum test. SPSS/PC+ (SPSS Inc., Chicago, IL) was used for these calculations. The stability of measurements of CBVR was estimated by calculating the mean difference and limits of agreement for paired estimations of CBVR, one pair before and one pair after L-NAME administration, in each animal (9).

RESULTS

Part of an experimental record, showing changes in [tHb] and MAP during an alteration in $PaCO_2$ and administration of 0.9% saline, L-NAME, and ACh, is shown in Figure 1. The effects of an injection of sodium nitroprusside are shown in Figure 2.

Measurements of PaO_2 in samples of arterial blood ranged between 8.09 and 22.7 (median 9.65) kPa. Rectal temperature ranged from 37.5 to 40.2°C (median 38.8), and blood glucose from 3 to 17 (median 9) mmol. There was no evidence of a systematic alteration in rectal temperature associated with L-NAME administration.

Effect of L-NAME. Changes in [tHb] and MAP associated with the injection of L-NAME and 0.9% saline are shown in Figure 3. The median (range) change from baseline in [tHb] after administration of 10 and 20 $mg \cdot kg^{-1}$ L-NAME were −0.59

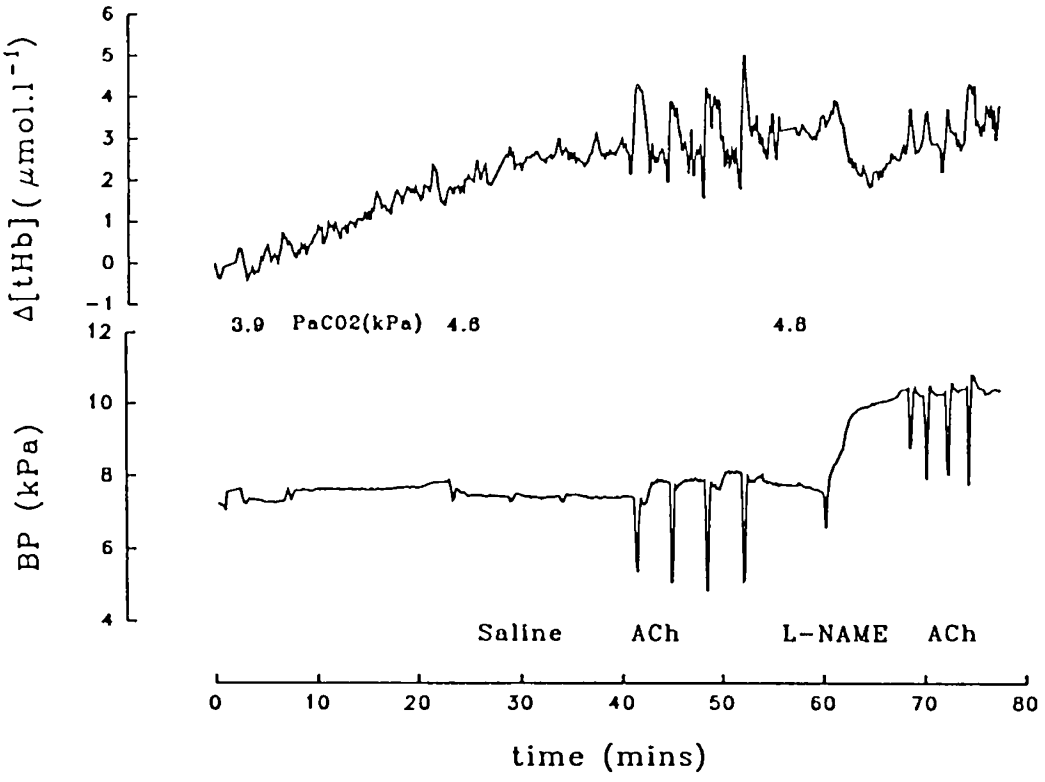

Fig. 1. Part of an experimental record from early in one experiment showing the effects of an alteration in Pa_{CO_2} and injections of 0.9% saline, ACh, and L-NAME on [tHb] and MAP (*BP*).

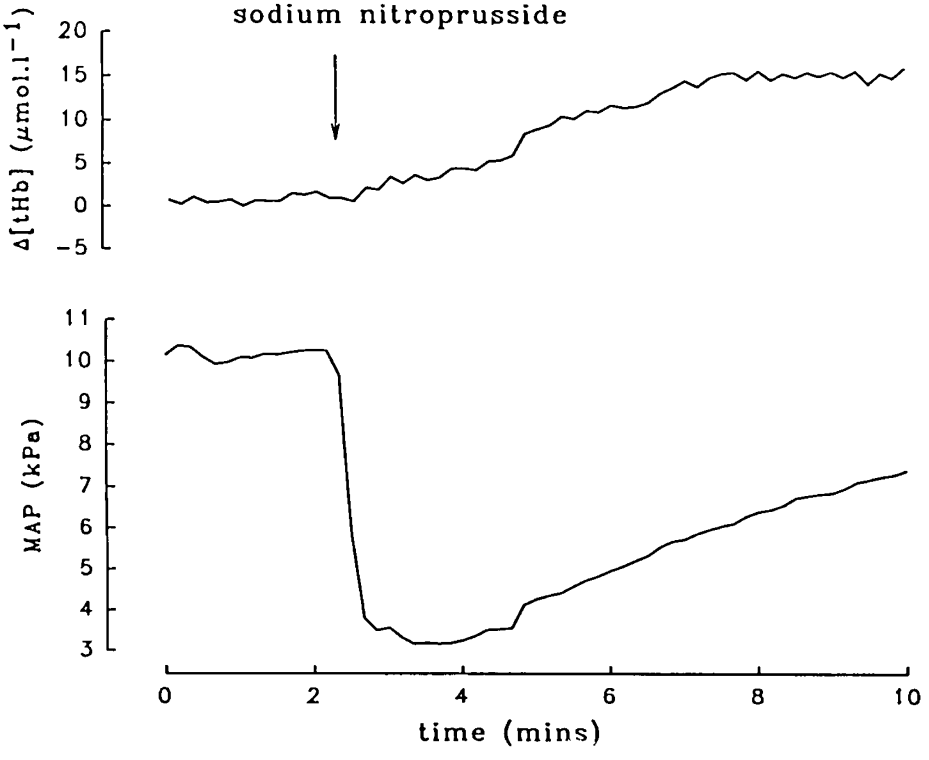

Fig. 2. Part of an experimental record showing the effect of sodium nitroprusside on [tHb] and MAP.

(−3.21 to −0.02) and −1.46 (−6.50 to −0.15) $\mu mol \cdot L^{-1}$, respectively; these changes, unlike those with subsequent injections of L-NAME, were significantly different from the alteration induced by 0.9% saline, which was 0.14 (−0.91 to 0.54) $\mu mol \cdot L^{-1}$ (Wilcoxon $p < 0.05$).

The median (range) changes of MAP from baseline after L-NAME administration at 10, 20, 30, and 100 $mg \cdot kg^{-1}$ were 2.3 (1.6 to 3.9), 0.8 (0.7 to 2.8), 0.3 (−0.5 to 0.9) and 0.5 (0.3 to 0.9) kPa, respectively, and all but the 30-mg dose were significantly different from the alteration induced by 0.9% saline, which was 0 (−0.2 to 0.25) kPa. (Wilcoxon $p < 0.01$).

Effect of ACh. ACh caused rapid but transient alterations in

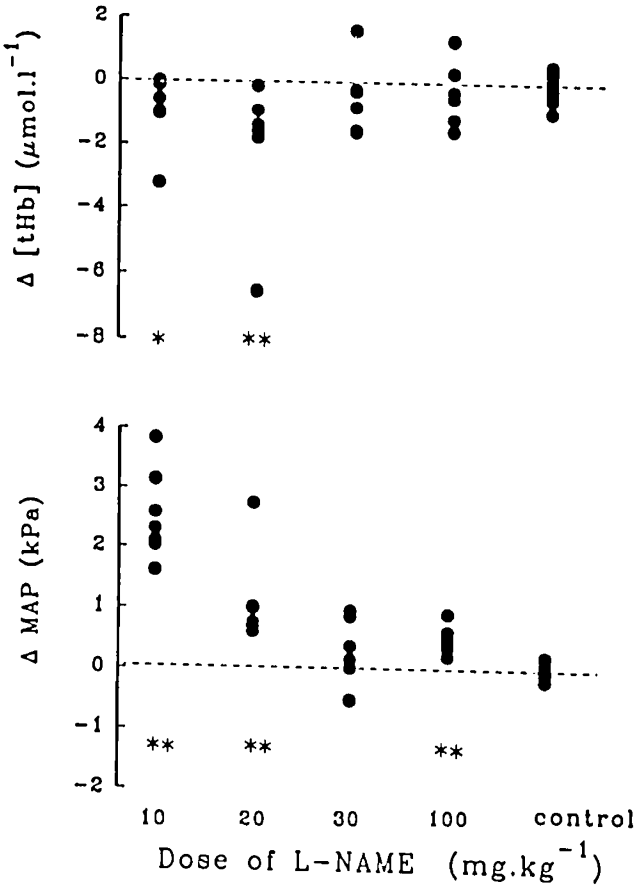

Fig. 3. Changes from baseline values of [tHb] and MAP after sequential doses of L-NAME or 0.9% saline (control). For L-NAME, 10 mg·kg^{-1}, $n = 7$; for 20, 30, and 100 mg·kg^{-1}, $n = 6$. Comparison with control ($n = 16$) by Wilcoxon: * = $p < 0.05$, ** = $p < 0.01$.

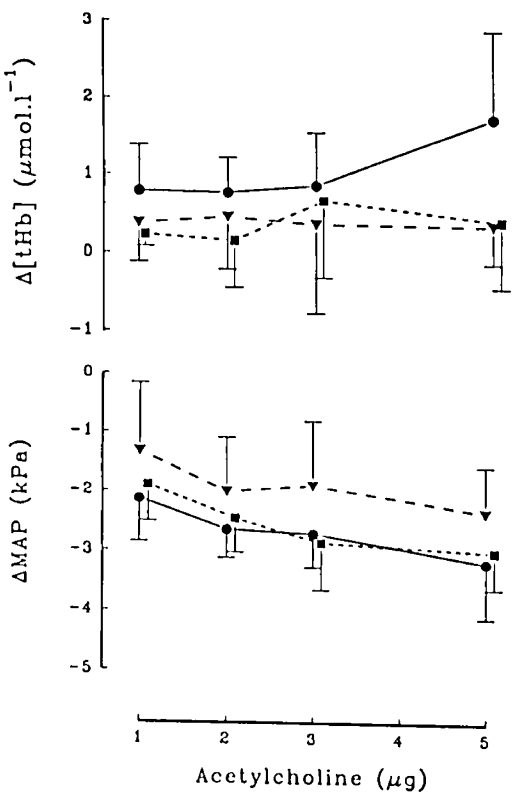

Fig. 4. Effect of L-NAME in attenuating the response of [tHb] and MAP to ACh. Before L-NAME (●), after 10 mg·kg^{-1} of L-NAME (■), and after 100 mg·kg^{-1} of L-NAME (▼). $n = 7$ except after 100 mg·kg^{-1} of L-NAME, where $n = 6$.

[tHb] and MAP and the dose-response relationships for these effects before and after administration of L-NAME are shown in Figure 4. ACh caused an increase in [tHb] and a fall in MAP that appeared to be dose-dependent and were attenuated by L-NAME (ANOVA $p < 0.05$).

Effect of L-NAME on CBVR. CBVR before and after administration of L-NAME is shown in Figure 5. The mean difference between repeated measures of CBVR was 0.1 μmol·L^{-1}·kPa^{-1}, and the limits of agreement were −2.1 to 2.4 μmol·L^{-1}·kPa^{-1}. Median (range) for average CBVR was 2.3 (0.2 to 4.6) before and 2.9 (0.1 to 14.1) μmol·L^{-1}·kPa^{-1} after L-NAME. There was no significant difference found between these groups by the Wilcoxon test.

Effect of sodium nitroprusside. The administration of sodium nitroprusside caused a sudden fall in MAP and a delayed rise in [tHb] (Fig. 2). The mean (SD) rise in [tHb] and fall in MAP were 13.44 (2.03) μmol·L^{-1} and 4.7 (1.6) kPa, respectively. Maximal effect on MAP occurred at a mean (SD) of 2.07 (0.50) min, and on [tHb] at 7.90 (3.10) min after injection. In most cases, MAP had begun to return toward baseline while [tHb] was still increasing.

NADPH-diaphorase staining. Heavy staining was seen in the endothelial cells of the carotid artery (Fig. 6*A*) and jugular veins as well as selected neurons and neuronal processes in the brain (Fig. 6*B*). The endothelial cells of cerebral vessels also stained, but less heavily (Fig. 6*B*).

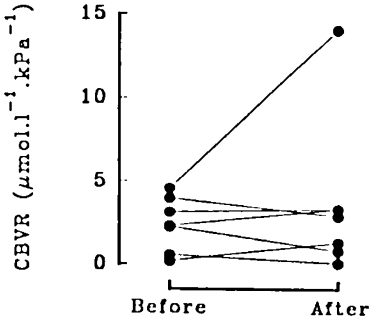

Fig. 5. CBVR before and after L-NAME in seven newborn piglets.

DISCUSSION

Accuracy of Measurements Using NIRS. The accuracy of the NIRS system for detecting changes in cerebral [tHb] depends on two factors: accurate data for the absorption spectra of the chromophores, and the validity of the modified Beer-Lambert relationship. Near-infrared spectra for Hb and cytochrome oxidase have been measured under carefully controlled conditions and recent studies have shown that the spectra measured *in vivo* and *in vitro* are identical (6, 10, 11). The validity of the modified Beer-Lambert law has been demonstrated by both theoretical modeling and experimental measurement in scattering media (7). System noise and systematic error have been shown to be a small fraction of the total near-infrared signal (12). One theoretical problem needs consideration: NO binds avidly to Hb, but no data are available concerning the optical properties of NO-Hb. It is possible that the near-infrared absorption spectrum of NO-Hb is significantly different from that of Hb; if so, and if NO-Hb concentration is altered, an extra mobile chromophore would be present in the tissue. However, because NO is constitutively released only in picomolar amounts (1), it can safely be assumed that an alteration in the quantity of NO-Hb would be undetectable by NIRS. Analysis of residuals from spectral fitting in our data has shown no evidence of an extra chromophore. NO-Hb is therefore very unlikely to have introduced errors into our data.

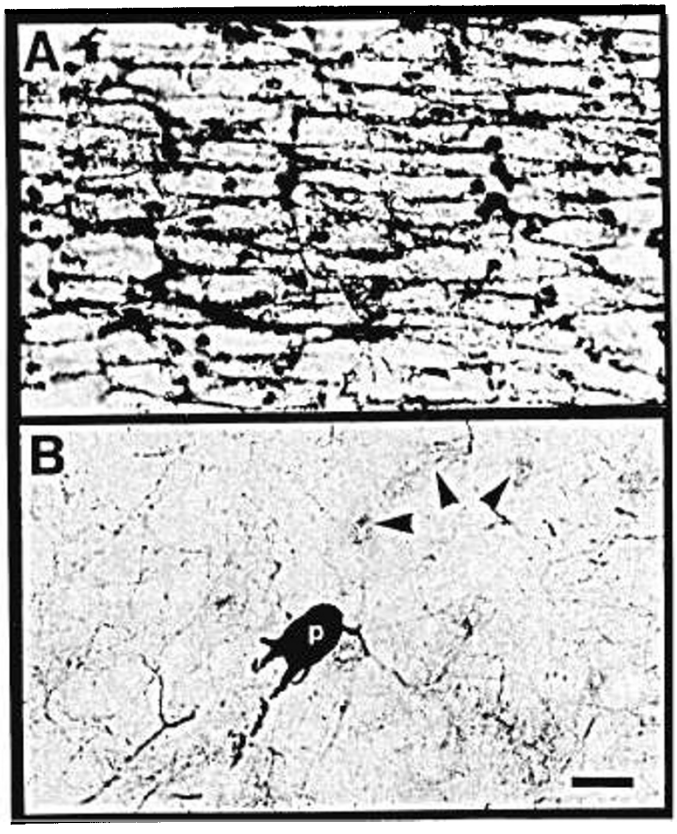

Fig. 6. Photomicrographs of carotid artery (*A*) and cerebral cortex (*B*) stained for NADPH-diaphorase. Staining is seen in a perikaryon (*p*), in nerve fibers and endothelial cells (*arrows*) in the cortex, and in endothelial cells in the carotid artery. The 1-cm bar denotes 30 μm.

Measurements of alterations in cerebral [tHb] do not distinguish between changes in arterial, capillary, or venous blood volumes, and we therefore cannot directly relate our findings to a particular cerebrovascular compartment. However, previous data have suggested that NO is a more potent physiologic mediator of arterial and arteriolar than of venous tone (1), and the alterations in MAP induced by L-NAME in the present study confirm that NO modulates vascular resistance in the newborn piglet. We made no observations of cerebral blood flow, but it is probable that changes in [tHb] are associated with changes in flow.

NO Synthase. There is evidence that NADPH-diaphorase and NO synthase are identical, and that NADPH-diaphorase staining may be used as a histochemical marker for NO synthase (13). Postmortem histochemical studies confirmed the presence of this enzyme in endothelial cells and neurons in newborn piglets. Blood vessels in most organs only express NO synthase in endothelium, whereas in the cerebral vessels of the adult rat it is also found in the nervous plexi of the adventitia (14). L-NAME inhibits NO synthesis in both neurons and endothelium (15), and neurogenic vasodilation of adult bovine cerebral artery rings *in vitro* is reduced by L-NAME (16). NO is also produced by neurons in the CNS and diffuses freely across membranes (15). We detected the enzyme at both endothelial and neuronal sites in newborn piglet brain, and although endothelium-dependent mechanisms probably account for the rise in MAP caused by L-NAME, at present it is not possible to exclude the involvement of a neurologic source of NO in the alterations in [tHb].

Effects of L-NAME. Inhibition of NO synthase. L-NAME was selected for these studies because it has been widely used to study the modulation of vascular tone by NO, and normally allows prolonged inhibition of NO synthesis because it is metabolized to L-nitro-arginine. The total dose of L-NAME (160 $mg \cdot kg^{-1}$) was chosen after consideration of dose-response data obtained from adult rats by Rees *et al.* (17), and it was administered in divided doses so that maximal inhibition could be observed. In view of the continued effect of L-NAME on MAP with the final dose, we cannot be sure that inhibition of systemic NO synthesis was complete and without partial recovery between doses. For this reason, we have related our data not to cumulative but to individual doses of L-NAME, although the results should not be interpreted as a dose-response relationship. However, the maximal change in [tHb] occurred with administration of 20 $mg \cdot kg^{-1}$ of L-NAME and no change was detected after subsequent injections; consequently, we are confident that basal cerebral NO synthesis was fully inhibited.

Basal NO secretion. L-NAME caused cerebral vasoconstriction and a rise in MAP. This implies that in the newborn piglet there is a baseline release of NO that maintains tonic vasodilation. Similar NO release has been noted in other experimental models although there is considerable heterogeneity between different vascular beds (1).

The median fall in [tHb] induced by L-NAME was no greater than $-1.46\ \mu m \cdot L^{-1}$. Although an absolute value for [tHb] was not measured, studies in newborn infants have shown it to be approximately $70\ \mu m \cdot L^{-1}$ (18). Inhibition of NO did not, therefore, appear to cause a large percentage fall in cerebral blood volume. Our results might be compared with experiments on adult rat aortic rings *in vitro*, where L-NAME alone induced approximately 5% of the maximal contraction caused by phenylephrine (17). They may be compatible with the suggestion that mechanisms mediated by NO may coordinate perfusion rather than determine total blood flow (19).

It is possible that the drugs used for anesthesia may have affected this result; halothane is a cerebral vasodilator and may reduce baseline NO production (20). However, the rate of clearance of halothane is 22% per minute (21) and at least 80 min were allowed for the effects of halothane to dissipate before the experiments were performed. It seems unlikely that this anesthetic significantly attenuated the effects of L-NAME.

ACh stimulation of NO secretion. Previous studies of the effect of ACh in the newborn piglet found that superperfusion of ACh over pial arteries led to vasoconstriction (2, 3). In the present study, however, ACh caused a rise in [tHb], demonstrating net vasodilation in the brain. The conflict between our results and previous reports may result from methodologic issues. Application of ACh by superperfusion to the surface of the brain may have stimulated contraction of cerebral vascular smooth muscle through muscarinic receptors (3) before diffusing onto the endothelium and causing NO release. The balance of these effects probably favored contraction over endothelium-mediated dilation.

L-NAME attenuated but did not abolish the rise in [tHb] caused by ACh, which implies that in the newborn piglet ACh-stimulated release of NO causes vasodilation, but that ACh also causes vasodilation by other mechanisms. Previous studies have shown that the hypotensive effect of ACh is only partially obliterated by inhibition of NO synthase (1). ACh is known to cause cerebral vasodilation by endothelium-dependent hyperpolarization (which blocks voltage-dependent calcium channels in smooth muscle cells) in rabbits (22) and to induce cerebral prostanoid synthesis in piglets (23).

Effects of sodium nitroprusside. The target for NO in vascular smooth muscle is a soluble guanylate cyclase, and binding leads to an increase in cGMP, which induces muscular relaxation (1). Sodium nitroprusside releases NO without enzymic catalysis, thus providing a pharmacologic stimulus to the NO transduction mechanism in smooth muscle cells (24). In the present study, sodium nitroprusside administration after L-NAME led to vasodilation, demonstrating that L-NAME had not affected vascular smooth muscle function. The effects of sodium nitroprusside on [tHb] were approximately an order of magnitude greater than those induced by L-NAME, which possibly reflects an action of exogenous NO on venous capacitance vessels, which are more

sensitive to exogenous nitrates than arteries. These results may suggest that the effect of L-NAME is predominantly on the arterial circulation (1).

Effect of L-NAME on CBVR. We found no evidence that NO was an important mediator of CBVR. Despite considerable heterogeneity within the group, the mean difference between repeated measurements of CBVR was small enough to give confidence in the stability of the method. Although we are unable to positively exclude a type II error, we have used similar methods to study newborn infants and showed that indomethacin caused a significant reduction in CBVR (25), implying that the technique should not have missed a large effect. One previous study, using laser-Doppler technology to study the cerebral surface in adult rabbits, has suggested that NO does mediate cerebrovascular responses to alterations in $PaCO_2$ (26). The conflict of results may be due to the species differences (which are very marked for prostanoid metabolism (27)), regional differences in cerebrovascular reactivity, maturational changes (8), or technical difficulties with the Doppler technique (28). It is also possible that barbiturate anesthesia may have affected CBVR, but we have not found evidence for this either in the literature or in other studies (Edwards AD, unpublished observation).

Differences between systemic and cerebral effects of L-NAME. Differences were apparent between the effect of L-NAME on the cerebral circulation and the mean blood pressure. The effect of L-NAME on [tHb] was maximal at 20 $mg \cdot kg^{-1}$, whereas MAP continued to increase with higher doses. Sodium nitroprusside caused an immediate fall in blood pressure, but a delayed rise in [tHb]; indeed, in several animals MAP was already returning toward baseline when [tHb] began to rise (Fig. 2). These observations may suggest differences in the role on NO in the hemodynamic control of the brain compared with the rest of the body.

Conclusion. NIRS is a simple, noninvasive method for studying the effects of NO *in vivo*. The present study demonstrated that NO maintained a basal dilator tone in the cerebral vasculature of the newborn piglet and that it mediated part of the vasodilatory effect of ACh, although whether the NO was derived from endothelium or from neurons was not clear. The effect of L-NAME on [tHb] was quantitatively small and NO was not an important modulator of CBVR. Additional studies using NIRS may help clarify the source of vasoactive NO in the newborn piglet brain and define differences between vascular beds *in vivo*.

Acknowledgments. The authors thank Professor G. Burnstock and Dr. F. Domer for many helpful discussions.

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