Endothelial Modulation of Contractions Caused by Oxyhemoglobin and $N^G$-Nitro-L-arginine in Isolated Dog and Monkey Cerebral Arteries

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Background and Purpose: Oxyhemoglobin is a key substance in provoking cerebral vasospasm and a scavenger of nitric oxide. The present study was designed to determine whether suppression of the action of endothelium-derived nitric oxide is involved in oxyhemoglobin-induced cerebroarterial contraction.

Methods: Dog and monkey cerebral artery strips with and without endothelium were immersed for isometric tension recording in modified Ringer-Locke solution aerated with 95% oxygen and 5% carbon dioxide.

Results: $N^G$-nitro-L-arginine, a nitric oxide synthase inhibitor, produced concentration-related contraction that was greater in the strips with intact endothelium than in those denuded of endothelium. The D-enantiomer caused no or only a slight contraction. In the presence of $N^G$-nitro-L-arginine, oxyhemoglobin elicited additional contraction that is comparable to or even greater than that obtained in the absence of the inhibitor. The oxyhemoglobin-induced contraction was attenuated by endothelium denudation.

Conclusions: Inhibition of the basal release of nitric oxide from endothelium results in dog and monkey cerebral arterial contraction. However, the inhibition of nitric oxide action is not a major mechanism involved in oxyhemoglobin-induced contraction; other mechanisms, such as the release of prostanoids, appear to be important. (Stroke. 1993;24:1584-1589.)

Key Words: • nitric oxide • oxyhemoglobin • dogs • monkeys

Vascular smooth muscle tone is controlled by vasoconstrictor and dilator factors that are generated from perivascular nerves, endothelium, and circulating blood. Imbalance of these factors by enhancing vasoconstriction and diminishing vasodilatation tends to provoke arterial spasm and increase arterial resistance in the brain and heart, thus exposing the patients to threat of death. Since the discovery of endothelium-derived relaxing factor (EDRF) by Furchgott and Zawadzki,1 much attention has been directed toward the role of the endothelium in protecting from the vasospasm.

Cerebral arteries isolated from primate and subprimate mammals respond to endogenous substances with relaxation by a mediation of EDRF or nitric oxide (NO); the substances include acetylcholine, substance P, arginine vasopressin, Bradykinin, thrombin, histamine, ATP, and ADP.2-10 Inhibitors of NO-synthase, such as $N^G$-monomethyl-L-arginine, $N^G$-nitro-L-arginine (L-NA), and L-NA methyl ester,11 suppress or abolish the relaxant response to these substances and are suggested to produce vascular contractions by eliminating the basal release of NO from the endothelium.12-14 Oxyhemoglobin (Hbo2) released from lysed erythrocytes is a key substance for provoking cerebral vasospasm after subarachnoid hemorrhage.15 Hbo2 scavenges EDRF/NO by binding to the heme group in the molecule,16 thus abolishing the ability of the endothelium to relax smooth muscle via the relaxing substance. Although such an action has been considered to be one of the mechanisms underlying cerebral vasospasm after hemorrhage, evidence for this hypothesis is lacking.

The present study aimed to determine whether the basal release of NO from the endothelium is involved in dilating isolated dog and monkey cerebral arteries under certain experimental conditions, and whether the elimination of NO action is a major mechanism underlying the Hbo2-induced contraction in the cerebral arteries. The data obtained from this in vitro study were compared with those from our previous studies in vivo.17,18

Materials and Methods

Mongrel dogs of either sex, weighing 8 to 15 kg, were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg) and killed by bleeding from the carotid arteries. The brain was rapidly removed, and basilar and middle cerebral arteries (0.5 to 0.8 mm outer diameter) were isolated. Branches of the superior mesenteric artery (0.6 to 0.9 mm) were also isolated. Additionally, basilar and middle cerebral arteries (0.4 to 0.7 mm) were removed from the brain of Japanese monkeys (Macaca fuscata) that had been killed by

Received December 21, 1992; final revision received April 8, 1993; accepted May 4, 1993.

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bleeding under ketamine (40 mg/kg IM) anesthesia. The arteries were helically cut into strips of approximately 20 mm long, with special care taken to reserve the endothelium. The specimens were vertically fixed between hooks in a muscle bath (20-mL capacity) containing the nutrient solution, which was aerated with a mixture of 95% O₂ and 5% CO₂ and maintained at 37±0.3°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer. The resting tension was adjusted to 1.5 g for dog arterial strips and to 1.0 g for monkey cerebral arterial strips, which are optimal for inducing the maximal contraction. Constituents of the solution were as follows (mmol/L): NaCl 120, KCl 5.4, CaCl₂ 2.2, MgCl₂ 1.0, NaHCO₃ 25.0, and dextrose 5.6. The pH of the solution was 7.34 to 7.41. Before the start of experiments, all of the strips were allowed to equilibrate in the bathing media for 60 to 90 minutes, during which time the fluid was replaced every 10 to 15 minutes.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph. The contractile response to 30 mmol/L K⁺ was first obtained, then the strips were washed three times with fresh media and equilibrated for 30 to 40 minutes. In the arterial strips partially contracted with prostaglandin (PG) F₂α (6% to 15% of contraction induced by 30 mmol/L K⁺), L-NA, Hbo₂, and methylene blue were successively applied, otherwise mentioned. Arterial strips with the intact and damaged endothelium were obtained from the same dogs, and paired comparisons were made in the responses to L-NA, Hbo₂, and methylene blue. The endothelium was removed by gently rubbing the intimal surface with a cotton pellet. Removal of the endothelium was determined by abolishment or marked suppression of the relaxation caused by substance P (10⁻⁸ mol/L), arginine vasopressin (10⁻⁷ mol/L), and ADP (10⁻⁷ mol/L) in the arteries partially precontracted with PGF₂α and also by the silver staining procedure.

The results shown in the text and figures are expressed as mean±SEM. Statistical analyses were made using Student's t test and Tukey's method after one-way analysis of variance. Drugs used were L-NA, NO₃-nitro-L-arginine (D-NA), substance P, arginine vasopressin (Peptide Institute, Mihon, Japan), L-arginine, methylene blue trihydrate, adenosine-5'-diphosphate (Nacalai Tesque, Kyoto, Japan), and PGF₂α (Ono Co, Osaka, Japan). Hbo₂ was prepared from dog hemoglobin (Sigma Chemical Co, St Louis, Mo) with the method described by Martin et al.¹⁶

Results

Response of Dog Cerebral Arteries

Responses to L-NA, Hbo₂, and methylene blue were compared in dog middle cerebral or basilar arterial strips with and without the endothelium, obtained from the same dogs. The strips were partially contracted with PGF₂α because no contraction was induced by L-NA under resting conditions (n=4). The addition of L-NA (10⁻⁴ and 10⁻³ mol/L) produced a concentration-related contraction in the endothelium-intact strips but did not significantly alter the arterial tone in the strips denuded of the endothelium (Fig 1, left). The difference in the values at 10⁻⁵ mol/L L-NA was statistically significant. Typical responses to L-NA in the strips with the intact and damaged endothelium are illustrated in Fig 2. Relaxations induced by 10⁻⁸ mol/L substance P in the endothelium-intact and -denuded strips were 60.3±4.0% and 20.1±5.5%, respectively (n=10, P<.001) and those by 10⁻⁷ mol/L vasopressin were 32.3±5.5% and 0.8±0.8%, respectively (n=10, P<.001). Increase in the concentration of L-NA to 10⁻⁴ mol/L did not produce additional contraction in three strips. L-Arginine (10⁻³ mol/L) reversed the contraction caused by L-NA. The mean value of the L-NA (10⁻² mol/L)–induced contraction was 100±23 mg (n=5), and the relaxation caused by L-arginine from the contracted level attained with the NO-synthase inhibitor averaged 118±42 mg (n=5). D-Arginine (10⁻³ mol/L) was without effect. D-NA (10⁻⁵ mol/L) did not produce contractions in the strips with and without the endothelium (n=5).

The strips exposed to L-NA (10⁻⁵ mol/L) responded to Hbo₂ (1.6×10⁻⁵ mol/L) with moderate contractions (Fig 1). Mean values induced solely by Hbo₂ in the strips with and without the endothelium were 22.9±4.6% and 12.1±2.8%, respectively (41.3±10.8% inhibition by endothelium denudation, n=10, P<.01), relative to those caused by 30 mmol/L K⁺. Methylene blue (10⁻⁵ mol/L) produced similar magnitudes of contraction in the endothelium-intact and -damaged strips; mean values were 24.7±7.2% and 20.3±3.7%, respectively (n=9, P<.05).

Oxyhemoglobin (1.6×10⁻⁵ mol/L) applied under resting conditions contracted the endothelium-intact and -denuded strips by 15.4±5.1% and 8.4±3.5%, respectively (38.0±5.2% inhibition by endothelium denudation, n=8, P<.001). In the strips contracted with Hbo₂, L-NA (10⁻⁵ mol/L) did not produce significant contrac-

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**Fig 1.** Line graphs show effects of NO₃-nitro-L-arginine (L-NA), oxyhemoglobin (OxyHb), and methylene blue (MB) on dog cerebral (left) and mesenteric (right) arterial strips with and without endothelium. The strips were partially contracted with prostaglandin F₂α; mean values in endothelium-intact and endothelium-damaged cerebral arteries were 102±20 mg and 128±18 mg (n=10), respectively, and those in mesenteric arteries were 86.7±11 mg and 116±28 mg (n=9), respectively. Contractions induced by 30 mmol/L K⁺ were taken as 100%; mean absolute values in intact and rubbed cerebral arteries were 1969±271 mg and 1765±349 mg (n=10), respectively, and those in mesenteric arteries were 2960±271 mg and 2608±326 mg (n=9), respectively. P<.02 (a) and P<.05 (b) compared with endothelium-intact strips. Vertical bars represent SEM.
tions (2.4±1.1%, n=8, in intact strips; 0.25±0.25%,
n=8, in rubbed strips).

Response of Dog Mesenteric Arteries

In mesenteric arterial strips with and without the
endothelium even when partially contracted with
PGF2α, the addition of L-NA (10⁻⁶ and 10⁻⁵ mol/L) and
Hbo₂ (1.6×10⁻⁵ mol/L) did not produce contractions
(Fig 1, right). On the other hand, the strips responded
to methylene blue (10⁻⁵ mol/L) with moderate contrac-
tions. Endothelium denudation did not influence the
effect of methylene blue. Relaxations induced by 10⁻⁶
mol/L acetylcholine in endothelium-intact and -dam-
aged strips were 56.3±6.2% and 8.4±3.5%, respecti-
vely (n=9, P<.001).

Response of Monkey Cerebral Arteries

The addition of L-NA in concentrations of 10⁻⁶ and
10⁻⁵ mol/L contracted the monkey middle cerebral or
basilar arterial strips in a concentration-dependent
manner. The contraction was significantly greater in
the endothelium-intact strips than in the strips denuded
of the endothelium (Fig 3). Relaxations induced by 10⁻⁷
mol/L ADP in the endothelium-intact and -damaged
strips were 74.5±4.0% and 12.5±2.7%, respectively
(n=6, P<.001). Typical tracings of the response to
L-NA are shown in Fig 4. D-NA (10⁻⁷ mol/L) produced
a slight contraction in the strips with endothelium
(11.4±3.1%, n=3).

In the strips contracted with L-NA (10⁻⁵ mol/L), Hbo₂
(1.6×10⁻⁵ mol/L) produced an additional contraction
(Figs 3 and 4). Mean values of the contraction in the strips
with and without endothelium were 44.2±4.9% and
29.5±3.9%, respectively (n=6, 31.2±10.1% inhibition by
endothelium denudation, P<.02). Methylene blue (10⁻⁵
mol/L) did not produce contraction but rather relaxed the
strips treated with L-NA and Hbo₂ (n=5).

Discussion

The addition of L-NA, an NO synthase inhibitor, produced
significant contractions of isolated dog and
monkey cerebral arteries with the intact endothelium.
The canine artery contraction was abolished by removal
of the endothelium or treatment with Hbo₂ and re-
versed by L-arginine. Similar contractions were also
observed in dog basilar artery rings stimulated by
N⁶-mononitro-L-arginine, in which endothelium de-
 nudation inhibited but did not abolish the contraction.22
The content of cyclic GMP in dog cerebral arteries is
markedly reduced by endothelium denudation (from
406 to 24.5 fmol/mg tissue weight).23 These findings
suggest that the induced contraction is associated with a
suppression of basal release of endothelium-derived
NO in arterial strips stretched to develop passive and

![Image of tracings](http://stroke.ahajournals.org/)

**Fig 2.** Tracings of typical responses to N⁶-nitro-L-arginine (L-NA), oxyhemoglobin (oxyHB, 1.6×10⁻⁷ mol/L), and methylene blue (MB; 10⁻⁵ mol/L) in middle cere-
bral artery strips obtained from the same dog, with intact and damaged endothelium. The strips were partially contracted with prostaglandin F₂α.

![Image of graph](http://stroke.ahajournals.org/)

**Fig 3.** Line graph shows effects of N⁶-nitro-L-arginine (L-NA) and oxyhemoglobin (OxyHB) on monkey cerebral arterial strips with and without endothelium. Strips were
partially contracted with prostaglandin F₂α; mean values in endothelium-intact and endothelium-denuded strips were 115±23 mg (n=8) and 102±25 mg (n=8), respectively.
Contractions induced by 30 mmol/L K⁺ were taken as 100%; mean absolute values in intact and denuded strips were 1145±180 mg and 1077±254 mg (n=8), respectively. P<.01 (a) and P<.02 (b) compared with endothelium-intact strips. Vertical bars represent SEM.
active tensions of 1.5 g. Increase in the release of relaxing factor(s) from the endothelium in response to mechanical stimuli, such as stretch, pressure, and shear stress, has been suggested. Similar results were also obtained in monkey cerebral arteries stimulated by L-NA, although the NO synthase inhibitor significantly contracted the arteries even denuded of the endothelium. The contraction caused by L-NA in the rubbed strips was greater than that seen in the intact strips stimulated by D-NA (24.5±6.8% versus 11.4±3.1%). Therefore, the contraction in the endothelium-damaged strips is due to incomplete removal of the endothelium or to mechanisms other than NO synthesis inhibition. In contrast to cerebral arteries, L-NA did not produce significant contractions in mesenteric arterial strips, suggesting that under the conditions used, NO in concentrations sufficient to relax the arteries is not released spontaneously from the endothelium.

Oxyhemoglobin contracted dog and monkey cerebral arteries even after the arteries were exposed to L-NA in a concentration (10^{-2} mol/L) in which cerebroarterial relaxations mediated by endogenous NO are almost abolished. This contraction is therefore considered to derive from a mechanism other than suppressed release of NO. The Hbo2-induced contraction in the presence and absence of L-NA was greater in the strips with the endothelium than in those denuded of the endothelium and are reportedly suppressed by treatment with cyclooxygenase inhibitors or PG receptor antagonists but not by a thromboxane A2 synthetase inhibitor. Production of PGF_2alpha and PGE_2 from isolated dog and monkey cerebral arteries is increased by Hbo2 or subarachnoid hemorrhage. It appears that the contraction is associated with the release of vasoconstrictor PGs mainly from the endothelium and also from subendothelial tissues. In contrast, Hbo2-induced contractions in cat cerebral arterial rings are not influenced by indomethacin. The discrepancy may be due to differences in animal species used (cat versus dog and monkey). According to Cocks et al., endothelin is liberated from cultured endothelial cells from the bovine aorta exposed for 4 to 24 hours to Hbo2-containing media. However, this is not likely to be the case in dog and monkey cerebral arteries, because the Hbo2-induced contraction rapidly developed (Figs 2 and 4) and attained a plateau within 30 minutes, during which time the endothelin-

[Diagram of Monkey Middle Cerebral Artery]

**FIG 4.** Tracings of typical responses to ADP (10^{-7} to 10^{-4} mol/L), NO7-nitro-L-arginine (L-NA; 10^{-6} and 10^{-3} mol/L), and oxyhemoglobin (oxyHb; 1.6×10^{-5} mol/L) in middle cerebral arterial strips obtained from the same monkey, with intact and damaged endothelium (E). Strips were contracted with prostaglandin F_{2alpha}; horizontal lines just left of right tracings represent the level before addition of prostaglandin F_{2alpha}. Contraction induced by 30 mmol/L K+ in endothelium-intact and endothelium-denuded strips were 860 mg and 832 mg, respectively. PA indicates 10^{-4} mol/L papaverine.
Intracisternal injections of L-NNA to anesthetized dogs significantly constrict basilar arteries, the effect being suppressed by treatment with hexamethonium.\(^1\) Therefore, the basilar arterial tone in vivo is postulated to be regulated by NO released from nitrooxidergic, vasodilator nerve terminals\(^2\) rather than NO from the endothelium. In contrast, under the experimental conditions used here, basal release of NO from the endothelium appears to participate in relaxing isolated cerebral arteries.

References
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Stroke. 1993;24:1584-1588
doi: 10.1161/01.STR.24.10.1584

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