Potentiation by Hypoxia of Contractions Caused by Angiotensin II in Dog and Monkey Cerebral Arteries

Kazuhide Yoshida, MD, PhD; Tomio Okamura, MD, PhD; and Noboru Toda, MD, PhD

**Background and Purpose:** Hypoxia alters the responsiveness to endogenous substances of cerebral arteries, possibly resulting in the modulation of blood supply to ischemic brain regions. The present study was undertaken to analyze the mechanism of potentiation by hypoxia of angiotensin II-induced cerebroarterial contractions.

**Methods:** Monkey and dog cerebral arterial strips with endothelium were suspended for isometric tension recording in Ringer-Locke solution aerated with 95% O₂–5% CO₂ (partial O₂ pressure, 570–600 mm Hg) or 95% N₂–5% CO₂ (approximately 10 mm Hg).

**Results:** Contractions induced by angiotensin II and substance P were potentiated by exposure to hypoxia, whereas contractile responses to prostaglandin F₂α were not influenced. Treatment with cyclooxygenase inhibitors abolished the peptide-induced contraction but did not alter the prostaglandin F₂α-induced contraction. Relaxations induced by arachidonic acid were suppressed by indomethacin and hypoxia, whereas those caused by a prostaglandin I₂ analogue were unaffected.

**Conclusions:** The potentiation by hypoxia of cerebroarterial contractions caused by angiotensin II and substance P appears to be due to an interference with the synthesis of prostaglandin I₂ from arachidonic acid and a resultant increase in the production of vasoconstrictor prostaglandins. (*Stroke* 1993;24:421–426)

**Key Words** • angiotensins • cerebral arteries • hypoxia • prostaglandins • dogs • monkeys

Angiotensin (Ang) II and substance P elicit contractions of dog and monkey cerebral arteries only when the endothelial cell function is retained. The contraction is abolished by aspirin, indomethacin, and prostaglandin (PG) receptor antagonists, suggesting that the release of vasoconstrictor PGs from the endothelium is involved. In the preliminary study, we found that the responses were potentiated under severe hypoxia.

Hypoxia contracts isolated human, monkey, dog, and sheep coronary arteries and sheep and rat pulmonary arteries. The mechanisms postulated are quite different: the release of vasoconstrictor PGs from subendothelial tissues or of vasoconstrictor substances other than PGs from the endothelium and the inhibition of basal release of vasodilator substances from the endothelium. Vasoconstrictor responses are inhibited or potentiated by hypoxia, depending on the agents used (norepinephrine, K⁺, and Ca²⁺ versus serotonin). Hypoxia or ischemia liberates many vasoactive substances or inhibits their synthesis in organs and tissues and thus modulates the vascular tone and local blood flow.

The aim of the present study was to determine the mechanism of the potentiating action of hypoxia on the contraction caused by Ang II and substance P in dog and monkey cerebral arteries with intact endothelium.

**Materials and Methods**

Mongrel dogs of either sex weighing 7–15 kg were killed by bleeding from the carotid arteries under pentobarbital anesthesia (50 mg/kg i.p.). Japanese monkeys (Macaca fuscata) of either sex weighing 6–10 kg were anesthetized with intramuscular injections of ketamine (25 mg/kg) and were killed by bleeding. The brain was rapidly removed, and basilar and middle cerebral arteries were isolated. The arteries were helically cut into strips approximately 20 mm long, with special care taken not to damage the endothelium. The specimen was vertically fixed between hooks in a muscle bath containing modified Ringer-Locke solution, which was maintained at 37±0.3°C and aerated with a mixture of 95% O₂–5% CO₂. 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 cerebral arterial strips and 1.0 g for the monkey arterial strips; these tensions are optimal for inducing the maximal contraction. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, CaCl₂ 2.2, MgCl₂ 1.0, NaHCO₃ 25.0, and dextrose 5.6. The pH of the solution was 7.38–7.41. Before the start of the experiments, all of the strips were allowed to equilibrate for 90–120 minutes in the bathing media, during which time the fluid was replaced every 10–15 minutes.
Isometric contractions were displayed on an inkwriting oscillograph (Nikon-kohden Kogyo Co., Tokyo). The contractile response to 30 mM K+ was first obtained, and the preparations were repeatedly washed and equilibrated. Only a single concentration of Ang II (10−7 M) or substance P (10−7 M) that produced maximal contractions in the arteries used was applied directly to the bathing media in each series. Preparations had been exposed for 15–20 minutes to the bathing media with 95% N2–5% CO2 (hypoxic media) or treated for approximately 20 minutes with blocking agents before the response to the peptides or other agonists was obtained. The partial oxygen pressure in the control solution (570–600 mm Hg) was decreased rapidly to approximately 10 mm Hg by exposure to the hypoxic media. Endothelial integrity was verified by a marked relaxation caused by 10−7 M Ca2+ ionophore A23187 (60–75% of relaxation caused by 10−4 M papaverine) and histologically by a silver staining method.

To measure the content of 6-keto-PGF1α in the bathing solution, cerebral arterial strips obtained from the dog brain were preincubated for 105 minutes for equilibration. A 15-minute incubation was performed after the preincubation. During the incubation, the strips were treated with or without Ang II under aerated and hypoxic conditions. The incubation medium was sampled. Amounts of 6-keto-PGF1α released from the cerebral arteries were measured with slight modifications of the method of Siess and Dray.16 Briefly, the incubation medium was adjusted to pH 3.5 with 1N HCl and, after addition of ethanol, was passed through a column of octadecylsilyl silica (Sep-Pak C18 cartridges, Waters Chromatography Division, Milford, Mass.). The column was washed with 15% ethanol and petroleum ether. Subsequent elution of the column with methyl formate gave a fraction of samples. The methyl formate was evaporated to dryness with a vacuum pump. The extract was reconstituted to 50 mM Tris-HCl buffer for measuring PGs. Each assay mixture containing the extract, anti-PG serum (Sigma Chemical Co., St. Louis, Mo.), and [3H]PG (>10,000 cpm, Amersham, Tokyo) was incubated at 4°C for 18 hours. Free and bound [3H]PGs were separated with the addition of dextrancoated charcoal, and the radioactivities of bound [3H]PGs were measured in a liquid scintillation counter.

Results shown in the text, figures, and table are expressed as mean±SEM. All reported n values refer to the number of animals studied. Statistical analyses were made using Student’s paired and unpaired t tests or Tukey’s method after one-way analysis of variance. Drugs used were PGE2, PGE3, (9,11),(11,12)-dideoxa-9,11-dimethylmethano-11,12-methano-13,14-dihydro-13-azoo-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epithrombaxone A2; (ONO3708, Ono Co., Osaka, Japan), Ang II, [Sar8, Ala10]Ang II (saralasin), substance P, 16-nitro-L-arginine (L-Na, Peptide Institute, Minoh, Japan), arachidonic acid, indomethacin, superoxide dismutase (Sigma), sodium (±)(1R,2R,3αS,8aR)-2,3,5α, 8b-tetrahydro-2-hydroxy-1-(E)-3(S)-3-hydroxy-4-methyl-1-oxet-6-ynyl)-1H-cyclopenta[b]-benzofuran-5-butyrato (beraprost sodium, Toray Industries, Inc., Tokyo), Ca2+ ionophore A23187 (Boehringer-Ingelheim, Ltd., Elmsford, N.Y.), and papaverine hydrochloride (Dainippon Co., Osaka, Japan).

**Figure 1.** Bar graphs showing modification by severe hypoxia (N2) and reoxygenation (O2, dotted bars) of the contractile response to angiotensin II (ANG II, 10−7 M, top panel) and prostaglandin F2α (PGF2α, bottom panel) of dog cerebral arterial strips with intact endothelium. Contraction in control media (O2, open bars) were taken as 100%; mean absolute values for experiments with ANG II and PGF2α were 313±65 mg (n=12) and 403±77 mg (n=10), respectively. Vertical bars represent SEM. *p<0.05 vs. control and reoxygenation by Tukey’s method.

**Results**

**Effect on Dog Cerebral Arteries**

The addition of Ang II in a concentration of 10−7 M produced a phasic contraction in dog cerebral arterial strips that was abolished by treatment with 10−7 M saralasin or 10−4 M indomethacin (n=5), as shown in an earlier report.1 In the strips exposed for 15–20 minutes to the bathing media aerated with 95% N2–5% CO2, the contraction caused by Ang II was significantly increased (Figure 1). However, contractions associated with PGF2α in concentrations (2−5×10−7 M) producing a magnitude of contraction similar to that induced by Ang II were not significantly increased by exposure to severe hypoxia. Typical recordings of the response to the peptide and PGF2α under control and hypoxic conditions are illustrated in Figure 2. The PGE2 (3×10−7 M)-induced contraction was decreased by hypoxia from 285±44.6 to 117±18.8 mg (62.7±3.0% decrease, n=6, p<0.001). In the same strips, Ang II–induced contractions were increased by 190±29.4% (n=6). As seen in normoxia, the contraction elicited by Ang II was markedly inhibited by indomethacin in hypoxic solutions (from 242±41 to 37±11 mg, n=4, 96.3±3.7% inhibition, p<0.001), whereas contractions induced by PGF2α and PGE2 were not altered.

In five of five strips, Ang II–induced contractions at partial oxygen pressures of approximately 600 mm Hg (control media) and 300 mm Hg did not differ. In addition, treatment with superoxide dismutase (20
units/ml) did not alter the peptide-induced contraction under control conditions \((n=6)\). Therefore, it seems unlikely that the increased production of oxygen radicals in control media depresses the contractile response to Ang II.

**Figure 2.** Tracings of typical responses to angiotensin II (ANG II, solid circles) and prostaglandin \(F_{2\alpha}\) (PGF\(_{2\alpha}\), open circles) of a dog basilar arterial strip with endothelium that has been exposed to control media and hypoxic media in the absence or presence of indomethacin (10\(^{-6}\) M).

Substance P (10\(^{-7}\) M)–induced contractions, susceptible to cyclooxygenase inhibitors,\(^2\) were also potentiated in the strips exposed to severe hypoxia, whereas the contraction induced by PGF\(_{2\alpha}\) was not significantly influenced (Figure 3). In the strips treated with 10\(^{-5}\) M L-NA, the substance P–induced contraction was also potentiated by hypoxia. The mean value of potentiation was 52.0±8.7\% \((n=8, p<0.001)\); the difference between the values in control and L-NA–treated strips was not significantly different.

In cerebral arterial strips treated with 10\(^{-7}\) M ONO3708, which is an antagonist of vasoconstrictor PGIs,\(^3\) and contracted with serotonin, arachidonic acid (10\(^{-5}\)–10\(^{-6}\) M) produced a concentration-related relaxation, which was markedly inhibited by treatment with 10\(^{-6}\) M indomethacin (Figure 4, right panel). Hypoxia significantly inhibited the arachidonic acid–induced relaxation (Figure 4, left panel). Concentration (10\(^{-6}\)–10\(^{-7}\) M)–relaxation response curves of beraprost, an analogue of PGI\(_2\), were not altered in hypoxic media; mean values of the relaxation under control and hypoxic conditions were 26.2±6.0\% and 25.3±5.5\% \((n=6)\), respectively, at 10\(^{-8}\) M beraprost and 71.0±5.9\% and 63.7±4.9\% \((n=6)\), respectively, at 10\(^{-7}\) M beraprost.

**Effect on Monkey Cerebral Arteries**

The addition of 10\(^{-7}\) M Ang II elicited a transient contraction of monkey cerebral arterial strips. Treatment with saralasin (10\(^{-7}\) M) and indomethacin (10\(^{-6}\) M) abolished or markedly suppressed the response to the peptide \((n=5);\) see Reference 1). The strips exposed to hypoxic bathing media responded to the peptide with a significantly greater contraction than that seen under normoxia (Figure 5). Contractions caused by PGF\(_{2\alpha}\)
Figure 5. Bar graphs showing modification by hypoxia (N2) of the contractile response to angiotensin II (ANG II, 10^-7 M; top panel) and prostaglandin F2α (PGF2α, bottom panel) in monkey cerebral arterial strips with endothelium. Contractions in control media (O2) were taken as 100%; mean absolute values in experiments with ANG II and PGF2α were 184±58 mg (n=9) and 258±98 mg (n=5), respectively. Vertical bars represent SEM. *p<0.05 vs. control.

Content of 6-Keto-PGF1α

The addition of Ang II (10^-7 M) to dog cerebral arterial strips significantly increased the content of 6-keto-PGF1α in the bathing medium (Table 1). Under hypoxic conditions, the stimulating effect of the peptide was suppressed.

**Table 1. 6-Ketoprostaglandin F1α Release From Dog Cerebral Arteries**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>6-Ketoprostaglandin F1α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>168±22.0</td>
</tr>
<tr>
<td>Ang II</td>
<td>6</td>
<td>405±75.2*</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7</td>
<td>178±64.9</td>
</tr>
<tr>
<td>Hypoxia + Ang II</td>
<td>7</td>
<td>235±77.9</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II. Values are mean±SEM for the number of preparations (n) from separate dogs. Paired comparisons were made in preparations under normoxia (control) and hypoxia and in preparations stimulated by 10^-7 M Ang II.

* p<0.05 vs. control.

**Discussion**

The present study revealed that contractions induced by Ang II in dog and monkey cerebral arteries and by substance P in dog cerebral arteries were potentiated under severe hypoxia, whereas the response to PGF2α or PG12 was not increased. These peptides produce the cerebral arterial contraction, possibly associated with the release of vasoconstrictor PGs, since the response is markedly reduced by treatment with cyclooxygenase inhibitors and PG receptor antagonists, such as ONO370813 and diphloretine phosphate. Our preliminary study with radioimmunoassay shows a stimulated release of PGF2α by Ang II from isolated dog cerebral arteries (K. Yoshida and N. Toda, unpublished data) in association with the release of 6-keto-PGF1α, a stable metabolite of PG12 (Table 1). These results indicate that the potentiation is ascribed to an increase in the production of vasoconstrictor PGs and a decrease in the release by the peptides of vasodilator substances or in the sensitivity to the vasodilators but not to the increased responsiveness to PGs. Reductions by hypoxia of the release of vasodilator prostanooids are postulated in sheep pulmonary arteries. Relaxations induced by arachidonic acid (10^-7 M) in dog cerebral arteries were abolished by 10^-6 M indomethacin (Figure 4, right panel), suggesting the involvement of vasodilator PGs, possibly PG12. In the cerebral arterial strips, only PG12 produces relaxation among the cyclooxygenase products used. In the arteries treated with ONO3708, the action of arachidonic acid was suppressed by hypoxia. Relaxant responses to beraprost, a stable PG12 analogue, were not influenced by hypoxia. These findings suggest an inhibition by hypoxia of the PG12 synthesis. Therefore, it appears that the potentiating effect of hypoxia on the contractile response to Ang II and substance P is due to an interference with the production of PG12 from arachidonic acid that would be liberated from activation of the peptide receptors. In fact, the content of 6-keto-PGF1α in bathing media, in which the arterial strips were stimulated by Ang II, was markedly reduced by hypoxia, suggesting a depression of PG12 synthesis. The impaired production of PG12 by hypoxia appears to shift the metabolism of arachidonic acid by cyclooxygenase to other prostanooids, such as PGF2α, PGE2, PGD2, and thromboxane A2. It has recently been demonstrated that hypoxia contracts...
monkey, human, dog, and sheep coronary arteries and has been suggested that the responses of the primate arteries are caused by cyclooxygenase products from the endothelium. Similar results were obtained in monkey cerebral arteries (N. Toda, K. Ayajiki, and T. Okamura, unpublished data). Hypoxic contraction in dog basilar arteries is postulated to result partly from a direct effect on smooth muscle as well as the endothelium.

In the case of substance P, inhibitions by hypoxia of the release of endothelium-derived relaxing factor may also be involved in the potentiation of contraction. However, this possibility would be minimal in dog cerebral arteries, since a similar potentiation by hypoxia was induced in response to Ang II, which does not have an ability to liberate endothelium-derived relaxing factor, and the potentiation of the response to substance P was seen to a similar extent (32% versus 52%) in the arteries treated with L-NA, a nitric oxide synthase inhibitor.

In dog cerebral arterial strips treated with ONO3708 and contracted with serotonin, PGF$_2$ alpha releases a production that is possibly due to the release of PGI$_1$ and its action on PGI$_1$ receptors. Hypoxia tended to potentiate the response to PGF$_2$ alpha, although the effect was not statistically significant. The contractile response to PGE$_2$, which does not liberate PGI$_1$ in an amount sufficient to relax cerebral arteries, was reduced by hypoxia. This contraction was a major effect of PGF$_2$ alpha in the concentrations used in the present study and was not influenced by indomethacin (Figures 2 and 6). Therefore, the relaxation caused by PGI$_1$ released is expected to be much less in the arteries stimulated by PGF$_2$ alpha than in those stimulated by Ang II or substance P when concentrations of PGF$_2$ alpha and peptides producing a similar magnitude of contractions are used.

Hypoxia vasoconstricts cerebral arteries and also potentiates the contractile response to endogenous vasoactive substances that are expected to stimulate phospholipase $A_2$ and liberate arachidonic acid; this potentiation could be a result of interference with the PGI$_1$ synthesis in the cerebroarterial wall and may be involved in the impaired blood supply to anoxic brain regions.

References

Potentiation by hypoxia of contractions caused by angiotensin II in dog and monkey cerebral arteries.

K Yoshida, T Okamura and N Toda

*Stroke*. 1993;24:421-425
doi: 10.1161/01.STR.24.3.421

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/24/3/421