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

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From the Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu, Japan.

Address for reprints: Dr. Noboru Toda, Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu 520-21, Japan.

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Isometric contractions were displayed on an ink-writing oscillograph (Nihon-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 acerated 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. Briefly, the incubation medium was adjusted to pH 3.5 with 1N HCl and, after addition of ethanol, was passed through a column of octadecysil 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 dextran-coated 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, PGE₁, (9,11), (11,12)-dideoxyl-9,11-dimethylmethano-11,12-methano-13,14-dihydro-13-azo-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epithromboxane A₂ (ON03708, Ono Co., Osaka, Japan), Ang II, [Sar¹, Ala⁶]Ang II (saralasin), substance P, 6-nitro-L-arginine (L-NNA, Peptide Institute, Minoh, Japan), arachidonic acid, indomethacin, superoxide dismutase (Sigma), sodium (±)-1(2R,2'2R,2'S,8βS)-1,2,3,5,8a,8b-tetrahydro-2-hydroxy-1-{[(E)-3'3'-3)-3-hydroxy-4-methyl-1-octen-6-ynyl]-1'H-cyclopenta-f[b]-benzofuran-5-butyrates (beraprost sodium, Toray Industries, Inc., Tokyo), Ca²⁺ ionophore A23187 (Boehringer-Ingerheim, Ltd., Elmford, 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. Contractions 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.](http://stroke.ahajournals.org/)

**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. 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 3.** Bar graphs showing modification by hypoxia \((N_2)\) and reoxygenation \((O_2\), dotted bars) of the contractile response to substance P \((10^{-7} \text{ M}, \text{top panel})\) and prostaglandin \(E_{2a}\) \((\text{PGF}_{2a}, \text{bottom panel})\) of dog cerebral arterial strips with endothelium. Contractions in control media \((O_2\), open bars) were taken as 100%; mean absolute values for experiments with substance P and \(\text{PGF}_{2a}\) were 624±90 mg \((n=11)\) and 416±61 mg \((n=6)\), respectively. Vertical bars represent SEM. *\(p<0.01\) vs. control and reoxygenation by Tukey’s method.

**FIGURE 2.** Tracings of typical responses to angiotensin II \((\text{ANG II}, \text{solid circles})\) and prostaglandin \(E_{2a}\) \((\text{PGF}_{2a}, \text{open circles})\) 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 \((10^{-6} \text{ M})\).

 Substance P \((10^{-7} \text{ M})\)-induced contractions, susceptible to cyclooxygenase inhibitors, were also potentiated in the strips exposed to severe hypoxia, whereas the contraction induced by \(\text{PGF}_{2a}\) was not significantly influenced (Figure 3). In the strips treated with \(10^{-5} \text{ M} \text{L-NA}\), 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 \(10^{-7} \text{ M} \text{ONO3708}\), which is an antagonist of vasoconstrictor PGIs\(^{13}\) and contracted with serotonin, arachidonic acid \((10^{-5}–10^{-6} \text{ M})\) produced a concentration-related relaxation, which was markedly inhibited by treatment with \(10^{-6} \text{ M} \text{indomethacin}\) (Figure 4, right panel). Hypoxia significantly inhibited the arachidonic acid−induced relaxation (Figure 4, left panel). Concentration \((10^{-6}–10^{-7} \text{ M})\)-relaxation response curves of beraprost, an analogue of PGL\(_3\), 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 \(10^{-8} \text{ M} \text{beraprost}\) and 71.0±5.9% and 63.7±4.9% \((n=6)\), respectively, at \(10^{-7} \text{ M} \text{beraprost}\).

**Effect on Monkey Cerebral Arteries**

The addition of \(10^{-7} \text{ M} \text{Ang II}\) elicited a transient contraction of monkey cerebral arterial strips. Treatment with saralasin \((10^{-7} \text{ M})\) and indomethacin \((10^{-6} \text{ M})\) 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 \(\text{PGF}_{2a}\)
tended to be increased, but the difference was not statistically significant. Typical responses are shown in Figure 6. In the hypoxic media, as in normoxia, indomethacin markedly suppressed the response to Ang II (from 350±101 to 32±7 mg, n=3, 90.3±1.2% inhibition, p<0.001) but not to PGF₂α.

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 (pg/mg tissue wt)</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>

* p<0.05 vs. control.

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.

**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 PGE₂ was not increased. These peptiades 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 dipheleoptin 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 PGI₂ (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 PGI₂. In the cerebral arterial strips, only PGI₂ 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 PGI₂ analogue, were not influenced by hypoxia. These findings suggest an inhibition by hypoxia of the PGI₂ 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 PGI₂ 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 PGI₂ synthesis. The impaired production of PGI₂ 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

**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.

**FIGURE 6.** Tracings of typical responses to angiotensin II (ANG II, 10⁻⁷ M) and prostaglandin F₂α (PGF₂α, 10⁻⁷ M) of a monkey basilar arterial strip with endothelium that was exposed to control and hypoxic media.
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. Arayaki, 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, PGF2α produces a relaxation that is possibly due to the release of PG1 and its action on PG1 receptors. Hypoxia tended to potentiate the response to PGF2α, although the effect was not statistically significant. The contractile response to PGF2α, which does not liberate PG1 in an amount sufficient to relax cerebral arteries, was reduced by hypoxia. This contraction was a major effect of PGF2α in the concentrations used in the present study and was not influenced by indomethacin (Figures 2 and 6). Therefore, the relaxation caused by PG1 released is expected to be much less in the arteries stimulated by PGF2α than in those stimulated by Ang II or substance P when concentrations of PGF2α 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 A2 and liberate arachidonic acid; this potentiation could be a result of interference with the PG1 synthesis in the cerebroarterial wall and may be involved in the impaired blood supply to anoxic brain regions.

References

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