Suppression of CO₂-Induced Relaxation of Bovine Retinal Pericytes by Angiotensin II

Takeshi Matsugi, Qian Chen, and Douglas R. Anderson

Purpose. To determine whether angiotensin II (Ang II), a vasoconstrictive peptide, changes the relaxation effect of elevated partial pressure of carbon dioxide (Pco₂) on pericytes.

Methods. The contractile tone of cultured bovine retinal pericytes was measured when the ambient Pco₂ was elevated in the absence or presence of Ang II. All experiments were performed in the bicarbonate-buffered solution at 37°C.

Results. Ang II (10⁻⁶ M) by itself did not increase the baseline tone of the pericytes. Raising the PCO₂ from 5% to 20% acidified the solution (pH dropped 0.51 ± 0.02 U) and caused a sustained and statistically significant 22.9% ± 4.6% relaxation of pericytes within 5 minutes (n = 8). In the presence of Ang II (10⁻⁶ M), the maximum relaxation induced by 20% Pco₂ was only 12.6% ± 4.5% (n = 6) at 3 minutes, and the relaxation was not sustained. The effect of Ang II was statistically significant. Pretreatment with the competitive Ang II receptor antagonist saralasin (10⁻⁶ M) for 10 minutes completely abolished the effect of Ang II (10⁻⁶ M) on the response of pericytes to 20% Pco₂. Saralasin by itself had no effect.

Conclusions. Ang II attenuated the relaxing response of pericytes to elevated Pco₂ through saralasin-sensitive Ang II receptors. Results suggest that some vasoactive agents, such as Ang II, could affect the pericyte responses to metabolic needs as signaled by local Pco₂. This experimental design may permit further investigation of the altered physiology of local blood flow regulation. Invest Ophthalmol Vis Sci. 1997;38:652-657.

Microvessels in the optic nerve head are primarily capillaries, and its wall is composed of pericytes and endothelial cells. Pericytes are particularly abundant in the optic nerve head and the retina. They contain contractile proteins¹⁻³ and contract or relax in response to vasoactive endothelium-derived factors, neurotransmitters, and hormones.⁴⁻⁹ Pericytes may participate in controlling local blood flow.

One of the local chemical mediators that change contractile tone of pericytes is carbon dioxide (CO₂). Cultured pericytes relax in response to an elevated partial pressure of CO₂ (Pco₂) and contract in response to a lowered Pco₂.¹⁰,¹¹ These responses correspond to the direction required to restore inadequate blood flow or to reduce overabundant perfusion in vivo. The results indicate that capillary pericytes probably contribute to the fine tuning of local blood flow through their contractile responses to local metabolic needs, such as CO₂ (metabolic autoregulation).

While studying the mechanism of glaucomatous optic nerve damage,¹²⁻¹⁴ we observed that a vasoconstrictor, angiotensin, affected intraocular pressure (IOP)-induced ischemic blockage of axonal transport in optic nerve.¹⁵⁻¹⁷ We hypothesized that the angiotensin might have reduced the ability of blood vessels in optic nerve head to respond to and compensate for the challenge to blood flow resulting from elevated IOP. Therefore, we examined in vitro whether angiotensin II (Ang II) affects the CO₂-induced relaxation of pericytes. Results of this study support the hypothesis that the ability of capillaries to regulate local blood flow can be altered by external influences.

MATERIALS AND METHODS

Cell Culture

Bovine eyes were obtained from Aries Scientific (Richardson, TX) and were shipped by overnight express
service on ice. We followed the method described by D'Amore\textsuperscript{18} to isolate pericytes. Under aseptic conditions, the retinas were removed from the eyes and transferred to a 100 mm diameter petri dish filled with sterile phosphate-buffered saline. The pigment epithelium and choroid were carefully removed. Retinas were rinsed thoroughly, minced, and incubated in phosphate-buffered saline containing 0.2% collagenase and 0.2% bovine serum albumin. After 1 hour of digestion, the minced retinas were filtered through a Spectramesh (111 \textmu m; Spectrum Medical Industries, Terminal Annex, LA), and the filtrate was pelleted by serial centrifugation (800g). The pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM) that contained 10% fetal bovine serum and was washed twice with the same medium. Resuspended cells were plated in the 75 mm\textsuperscript{2} flasks with DMEM containing 10% fetal bovine serum, 50 mg/ml fungizone, and 1.25 mg/ml gentamicin. After approximately 3 weeks, the cells would grow into a near-confluent stage and would be transferred to 60 mm diameter petri dishes, the bottoms of which were coated with silicone (dimethylpolysiloxane; 60,000 cp viscosity; Sigma, St. Louis, MO). The silicone-coated dishes had been flamed to create an elastic solidified surface and were sterilized by ultraviolet radiation overnight. All the experiments were performed 3 to 5 days after this first passage. We had previously identified and characterized the cultured cells as pericytes.\textsuperscript{19}

**Measurement of Contractile Tone of Pericytes**

The method to observe the contractile tone of cells cultured on the silicone surface was developed originally by Harris and coworkers.\textsuperscript{20} In the system, when pericytes are cultured on a silicone surface, the tension produced by the cells compresses the silicone substrate into visible wrinkles under a phase-contrast microscope (Fig. 1). This system has made it possible to evaluate contraction and relaxation of pericytes in detail. In each of our experiments, the length of each

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933197/)
clearly discerned wrinkle beneath the several cells in the microscopic field was measured, and the sum of all the lengths was calculated with a digitizer (Video Plan 2; Zeiss, Thornwood, NY). The two ends of a wrinkle were determined by the contrast difference from the background on the picture, as shown in Figure 1. These images were analyzed in a blind manner by the same observer through all analyses.

We report our results of the contractile tone in terms of the summed total length of the wrinkles, 1, caused by the cells. The 1 at the beginning of the experiment is designated as 10. Thus, the ratio 1/10 represents the change in contractile state from the spontaneously contracted condition at the beginning of the experiment. A ratio more than 1 represents contraction, and a ratio less than 1 represents relaxation.

All experiments were performed in the bicarbonate-buffered solution at 37°C on the stage of an inverted phase-contrast microscope with a solid-state TV camera (Panasonic, Osaka, Japan) and a video printer (Mitsubishi, Cypress, CA). The culture dish was placed in a specially designed chamber in which the temperature and the ambient gas condition were controlled.

The DMEM culture medium was exchanged for the bicarbonate-buffered solution equilibrated with 5% PCO2. After 20 minutes, the solution was exchanged for bicarbonate-buffered solution equilibrated with 20% PCO2. Previously, we had found that an increase in PCO2 from 5% to 10% or 20% produced a dose-dependent relaxation. In the Ang II experiments, Ang II at 10^-6 M was added 10 minutes before the exchange for the solution equilibrated with 20% PCO2. When saralasin (1-Sar-5-Val-8-Ala-angiotensin II) was needed, it was added 10 minutes before the addition of Ang II. The pH of each solution was monitored, and neither Ang II nor saralasin had an effect on pH (Table 1).

### Chemicals and Solutions
Phosphate-buffered saline, DMEM, fetal bovine serum, fungizone, and gentamicin were purchased from Life Technologies (Gaithersburg, MD). Collagenase, bovine serum albumin, Ang II, and saralasin were purchased from Sigma. Ang II and saralasin were dissolved in double-distilled water as a stock solution and stored at −20°C until use. The constituents of bicarbonate-buffered solution were as follows: NaCl, 126.3 mM; NaHCO3, 14.7 mM; KCl, 5 mM; MgCl2, 1 mM; CaCl2, 1.5 mM; glucose, 10 mM.

### Data Analysis
All data were expressed as the mean ± standard error. The number of experiments (n) represents the number of times the experiment had been repeated. Statistical significance was determined by an unpaired Student's t-test at a designated time. Results were analyzed with repeated measures analysis of variance of the overall group means.

### Results
Elevation of PCO2 Caused Pericytes to Relax
Raising PCO2 from 5% to 20% acidified the bicarbonate-buffered solution (Table 1) and caused significant relaxation of the pericytes within the first minute after the solution exchange (Figs. 1B, 2). The relaxation was 22.9% ± 4.6% at 5 minutes, and it persisted as the high ambient PCO2 was maintained. Pericytes showed little change in their contractile tone when the solu-
Effects of CO₂ and Angiotensin on Pericytes

Ang II Reduced the Relaxation of Pericytes

Effects of CO₂ and Angiotensin on Pericytes

Ang II (10⁻⁶ M) by itself did not increase the baseline tone of pericytes in culture, affects the responsiveness of pericytes to metabolic conditions as signaled by local PCO₂. The result implies that under certain circumstances, Ang II could reduce the capacity of metabolic autoregulation of microvascular walls to CO₂.

In general, the contractile state of cells, such as smooth muscle cells and pericytes, is regulated by intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) and the sensitivity of myofilaments to Ca²⁺. Elevation of PCO₂ in the extracellular medium relaxes cultured pericytes and acidifies the extracellular medium and the cytoplasm of the cells. The decrease in extracellular and intracellular pH have been reported to reduce [Ca²⁺]ᵢ or to decrease the sensitivity of myofilaments to Ca²⁺. Therefore, an awaiting experimental confirmation is the assumption that there is a reduced [Ca²⁺]ᵢ, or a reduced sensitivity of myofilaments to Ca²⁺ to explain the CO₂-induced relaxation of pericytes. Ang II increases [Ca²⁺]ᵢ by increasing Ca²⁺ influx through calcium channels and by releasing intracellular Ca²⁺ from sarcoplasmic reticulum. Based on our current experiments, we are not able to discern the mechanisms underlying the interaction between the effects of Ang II and CO₂. Further experiments on [Ca²⁺]ᵢ in pericytes will lead to a better understanding of the interactive mechanisms, in particular if Ang II alters the sensitivity of CO₂ to [Ca²⁺]ᵢ, alters the sensitivity of the contractile myofilament to the [Ca²⁺]ᵢ, or both.

The 10⁻⁶ M Ang II used in the experiments may or may not be the same as levels in the interstitial fluid near pericytes in vivo. Although 10⁻⁶ M Ang II is higher than the concentration in human plasma reported by other researchers, it is possible that pericytes are exposed to higher concentrations of Ang II than they are in plasma because Ang II can be locally produced and released by local tissues, including ocular tissues, in addition to the circulating Ang II in blood. There is evidence that high levels of angioten-
sin-converting enzyme exist in retina and microvascular endothelial cells. Further experiments of this type will permit more detailed understanding of altered contractile physiology and will addressing such questions as: Do the competing influences occur in vascular smooth muscle of arteries? Do vasoconstrictors other than Ang II in active or subliminal doses limit reactivity to CO2? Do Ang II or other vasoconstrictors limit the responses to other physiologic indicators of vascular underperfusion (such as hypoxia or adenosine) and limit the response to all vasodilators? Do subliminal doses of vasodilators enhance the effectiveness of physiologic vasodilators that participate in autoregulation of local blood flow? As recently reviewed elsewhere, the reduction of responsiveness of blood vessels to metabolic autoregulatory vasodilators may have pathophysiologic relevance to glaucoma as well as to other diseases, and understanding the methods to restore autoregulatory responses may have therapeutic application.

**Key Words**
angiotensin II, autoregulation, carbon dioxide, glaucoma, pericytes

**Acknowledgments**
The authors thank William Feuer for graciously assisting with the statistical analysis.

**References**


