 Comparative Effects of Angiotensin-(1-7) and Angiotensin II on Piglet Pial Arterioles

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Background and Purpose: Recent investigations indicated that degradation fragments of angiotensins could be involved in the regulation of the cerebral circulation and that their effects might be mediated by prostaglandins. The present study was designed to examine the effect of angiotensin-(1-7), a major endogenous heptapeptide fragment, on cerebral arteriolar diameter and compare it with the octapeptide angiotensin II, and further to determine whether prostaglandins mediate their effects.

Methods: Newborn, anesthetized pigs were equipped with a closed cranial window, and the diameter of one pial arteriole was measured using intravital microscopy.

Results: Topical application of angiotensin-(1-7) (n=9) increased the diameter by 6.8±5.3% (mean±SEM), 10.4±5.2%, 14.3±5.9%, and 17.5±7.7% (P<.05) at 10-7, 10-4, and 10-4 mol/L, respectively (baseline, 94±3 μm). Topical application of angiotensin II (n=8) increased the diameter by 9.6±7.8%, 9.6±7.6%, 11.3±8.4% (P<.05), and 5.5±7.9% at 10-7, 10-4, 10-3, and 10-4 mol/L, respectively (baseline, 94±5 μm). After administration of indomethacin (5 mg/kg IV), which did not significantly change the baseline arteriolar diameter, neither angiotensin-(1-7) at 10-4 mol/L nor angiotensin II at 10-3 mol/L caused significant vasodilation.

Conclusions: The results indicate that angiotensin-(1-7) is a modest dilator in the cerebral circulation, as is angiotensin II, and that prostaglandins may mediate responses. (Stroke. 1993;24:2041-2045.)

Key Words • angiotensins • cerebral circulation • prostaglandins • pigs

It has been found that angiotensins, including those circulating in blood and those produced in the brain vasculature, play a role in the regulation of the cerebral microcirculation.1,2 Recent investigations revealed that the degradation fragments of angiotensins could be also involved in this regulation.3-4 Angiotensin-(1-7) [Ang-(1-7)], a major endogenous heptapeptide fragment produced in amounts equal to or greater than the octapeptide angiotensin II (Ang II) in the brain,5-7 has been found to be similar to Ang II in some biologic activities such as causing hypotension and bradycardia.8-9 exciting neurons,9 and stimulating vasopressin release from neurohypophysial explants.10 Further, other studies suggested that the effects of angiotensins and their other degradation fragments on cerebral circulation may be mediated by prostaglandins,11-13 and Ang-(1-7) increased prostaglandin (PG) E2 synthesis in vasa deferentia with a potency equivalent to that of Ang II.14 From the above findings, it could be hypothesized that Ang-(1-7) may play a role in the regulation of cerebral arteriolar diameter, as apparently Ang II does, and its effect may be mediated by dilator prostaglandins. The purposes of the present study were (1) to examine the effect of Ang-(1-7) on cerebral arteriolar diam-

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ter and compare it with Ang II and (2) to determine whether prostaglandins mediate these effects.

Materials and Methods

Seventeen newborn piglets of either sex weighing 1.2 to 2.0 kg were anesthetized with sodium thiopental (30 mg/kg IP) and then α-chloralose (75 mg/kg IV). Supplemental doses of α-chloralose were injected to maintain the required level of anesthesia. The piglet was intubated and artificially ventilated with room air. A femoral artery and vein were cannulated with PE-90 tubing. Arterial blood samples were taken regularly from the femoral artery to measure blood gases, and the Pco2, pH, and Po2 were maintained within the normal physiological range. The rectal temperature was maintained at 37±1°C with a water circulation heating pad and a digital thermometer.

The piglet's head was fixed in a stereotaxic apparatus, the scalp was cut, and the connective tissue over the parietal bone was removed. Small hemorrhagic holes in the bone were sealed with bone wax, and an opening 19 mm in diameter was made in the left parietal bone. The dura was cut and reflected over the skull. As previously described,15 a stainless steel and glass cranial window with three ports was put into the opening, sealed with bone wax, and cemented with dental acrylic. The closed window was filled with artificial cerebrospinal fluid (aCSF) that was warmed to 37°C and equilibrated with 6% O2/6.5% CO2 in N2. The composition of the aCSF (mmol/L) was: KCl 2.9, MgCl2 1.4, CaCl2 1.2, NaCl 132, NaHCO3 24.6, urea 6.7, and glucose 3.7. The microves-
sels on the cerebral surface were observed under a microscope (Wild M7 S, Switzerland) equipped with television camera (Panasonic, Japan) and a monitor (Panasonic). The diameter of the blood column in arterioles was measured perpendicularly on the monitor with a video microscler (VAP-1000, For-A Co, Newton, Mass). The magnification of the objective lens was \( \times 18 \), and the final magnification of the vessel on the monitor screen was \( \times 450 \).

The cranial window was gently flushed with aCSF several times until a stable baseline was obtained. Then we infused the window in turns with \( 10^{-7}, 10^{-6}, 10^{-5} \), and \( 10^{-4} \) mol/L of Ang-(1-7) or Ang II (both obtained from Sigma Chemical Co, St Louis, Mo) in aCSF and recorded the change in diameter for each minute over a 5-minute period at each concentration. Then the cerebral surface was flushed with aCSF. Indomethacin dissolved in saline was injected intravenously (5 mg/kg). This dose of indomethacin reduces resting CSF values toward nondetectable levels and abolishes or greatly reduces increases in response to various stimuli. The change in diameter at 10, 20, and 30 minutes after the injection was recorded, and Ang-(1-7) or Ang II was applied again at the above concentrations and time intervals.

All values are expressed as mean±SEM. A repeated-measurements analysis of variance was used to test the statistical difference among the data groups, followed by the Student-Newman-Keuls test where appropriate. A value of \( P<.05 \) was considered statistically significant.

**Results**

**Effects of Angiotensin-(1-7) and Angiotensin II on Cerebral Arteriolar Diameter**

As shown in Fig 1, at 3 minutes after the topical application of Ang-(1-7), the cerebral arterioles dilated by 14.3±5.9% and 17.5±7.7% at the concentrations of 10\(^{-5}\) and 10\(^{-4}\) mol/L, respectively. At 10\(^{-4}\) mol/L, the dilation showed a significant difference from baseline \( (P<.05) \). The baseline diameter was 93.9±3.2 \( \mu \text{m} \) (mean±SEM) \( (n=9) \). Of the 9 arterioles studied, 8 dilated at all doses, and 1 moderately dilated at 10\(^{-7}\), 10\(^{-6}\), and 10\(^{-5}\) mol/L and constricted at 10\(^{-4}\) mol/L. If we exclude the last piglet, the dilation at 10\(^{-4}\) mol/L is 21.7±7.3%. Arterial blood pressure during baseline was 60±5 mm Hg and did not change during application of Ang-(1-7).

The topical application of Ang II also caused dilation by 11.3±8.4% and 5.5±7.9% at the concentrations of 10\(^{-5}\) and 10\(^{-4}\) mol/L, respectively. At 10\(^{-5}\) mol/L, the dilation showed a significant difference from baseline \( (P<.05) \). The baseline diameter was 94.3±5.4 \( \mu \text{m} \) (mean±SEM) \( (n=8) \). Of the 8 arterioles studied, 7 dilated and 1 constricted mildly after the application of Ang II. The arterial blood pressure did not show a significant change after the application of Ang-(1-7) or Ang II at 10\(^{-7}\) to 10\(^{-5}\) mol/L. At 10\(^{-4}\) mol/L Ang II tended to increase the pressure from 63±4 (baseline) to 73±8 mm Hg, which was not significantly different.

**Effects of Angiotensin-(1-7) and Angiotensin II on Arteriolar Diameter in Indomethacin-Treated Piglets**

At 30 minutes after intravenous administration of indomethacin, the arteriolar diameter did not show a significant change, and topical application of Ang-(1-7) at 10\(^{-4}\) mol/L \( (n=8) \) or Ang II at 10\(^{-5}\) mol/L \( (n=7) \) no longer caused significant dilation in cerebral arterioles (Fig 2). The arterial blood pressure also did not show a significant change during the above treatments. Baseline arterial blood pressure was 61±3 mm Hg for the Ang-(1-7) group and 64±4 mm Hg for the Ang II group. At 10\(^{-5}\) and 10\(^{-4}\) mol/L, Ang II tended to increase the arterial blood pressure to 76±8 and 81±9 mm Hg.
respectively, but neither of the increases was statistically different from baseline.

Discussion

The major findings in the present study are that (1) Ang-(1-7) can cause mild dilation of cerebral arterioles in newborn piglets, as does Ang II, and (2) prostaglandins may mediate the vasodilation induced by Ang-(1-7) or Ang II.

The maximum vasodilation induced by topical application of Ang-(1-7) was 17.5% at 10⁻⁴ mol/L, and the dilation showed a dose-dependent tendency. The maximum dilation induced by Ang II was 11.3% at 10⁻⁵ mol/L, and the dilation was only 5.5% at 10⁻⁴ mol/L with a modest but not significant change in arterial pressure. The results indicate that Ang-(1-7) can cause mild dilation of cerebral arterioles, as does Ang II, and the dilating effect of Ang-(1-7) may be dose dependent and may be stronger than that of Ang II at the 10⁻⁴ mol/L concentration.

Under normal and stressful conditions, both the circulating angiotensins and the locally produced angiotensins in the brain vasculature participate in the regulation of cerebral arterioles. However, it is hard to estimate local concentrations of angiotensins for comparison to the doses that we applied in our experiments because the metabolism of angiotensins is very fast. For example, the half-degradation time of Ang II in blood is only 12.7 seconds. Therefore, the local concentration of angiotensins around the cerebral arterioles remains unknown because the ex vivo measured angiotensin may not reflect the true local concentration. In our experiments, we used the concentrations of 10⁻⁷ to 10⁻⁴ mol/L in comparison with 10⁻⁷ to 10⁻³ mol/L and 10⁻¹⁰ to 10⁻⁵ mol/L in the experiments of Haberl et al. with Ang II. Haberl et al. were able to show that exogenous renin was able to cause an effect similar to that of Ang II at 10⁻⁵ mol/L. Therefore, levels of angiotensins around cerebral arterioles can reach the vasoactive range. Our results could provide a useful estimation for the further investigation for the effects of the angiotensins and their fragments under normal and pathophysiological conditions. Although the increase in arteriolar diameter was modest, angiotensins could have a major effect on cerebrovascular resistance. A 17.5% increase in diameter in an arteriole of 93.9 μm would result in a 48% decrease in vascular resistance, which could increase CBF.

Our observation of Ang II-induced cerebral vasodilation is consistent with the vasodilation and decrease in small vessel resistance reported in rat and rabbit. The maximum mean dilation by 10⁻⁷ mol/L Ang II in our experiments (11.3%) was smaller than those found by Haberl et al. (approximately 17% in rats and 21.6% in rabbits). The reasons might be that we used different species and that the vessel size we observed (94±5 μm in diameter) was bigger than in their studies (37±4 and 67±4 μm).

We did not see the significant vasoconstriction induced by Ang II that has been found in hamster or in cat. These conflicting results might represent species variability. However, in our experiments one arteriole showed mild constriction during the 10⁻⁴ mol/L Ang-(1-7) application, and one arteriole constricted mildly during Ang II application at all concentrations. This kind of phenomenon was also found in rabbit. From these results, it could be speculated that, in addition to species differences, there may be other reasons for the conflicting responses to Ang II or Ang-(1-7). First, the receptors for Ang II are heterogeneously located in the brain. Ang-(1-7) was also found to be heterogeneously distributed in the brain. Therefore, Ang II and Ang-(1-7) might cause different responses in different vessels. Second, Ang II has been shown to release both vasoconstrictor and dilator prostaglandins from cerebral arteries. Therefore, it is possible that Ang II or Ang-(1-7) could cause either vasodilation or vasoconstriction in different vessels or under different conditions. Third, if Ang II induced constriction of large arteries, compensatory dilation of downstream arterioles might occur. Fourth, when Ang II or Ang-(1-7) acts on target cells, it may affect different second messenger systems such as cyclic adenosine monophosphate or cyclic guanosine monophosphate and result in different responses.

Many investigations suggested that Ang II-induced vasodilation may be mediated by prostaglandins. Some reports suggested that the activated receptors linked to phospholipase A₂ resulted in the release of arachidonic acid from cell membranes and the synthesis and release of PGI₂. The PGI₂ caused dilation of vascular smooth muscle. This hypothesis was supported by the evidence that a PGI₂-like substance was detected in superfusate of renal arteries in response to Ang II, and the relaxation of cerebral arteries (in vitro) induced by Ang II was reversed to a contraction by aspirin and indomethacin and suppressed by 15-hydroxyperoxarachidonic acid and tranylcypromine, inhibitors of PGI₂ synthesis, or by dexamethasone, an inhibitor of phospholipase A₂. Other experiments showed that Ang II increased the PGI₂ (measured as 6-keto-PGF₁α) release from dog renal arteries, Ang II could stimulate PGE₂ production in mesenteric artery that was blocked by indomethacin, and Ang II-induced cerebral vasodilation in rat was blocked by topical application of indomethacin. It has been suggested that the vascular endothelial cells may be the major site to produce PGI₂ or PGE₂ while PGE₂ is mainly produced in other cell types, perhaps vascular smooth muscle cells. Thus, probable sources of dilator prostaglandins in piglet pial arterioles could be endothelial and smooth muscle cells.

Previous experiments indicate that indomethacin, a cyclooxygenase inhibitor, is a relatively specific inhibitor of cerebral arterial dilation. For example, the intravenous injection of indomethacin in piglet did not affect lipoxin-induced cerebral vasodilation or N-methyl-D-aspartate (NMDA)–induced cerebral vasodilation. The mechanism of NMDA-induced pial arteriolar dilation is apparently via the nitric oxide synthetic pathway. Also, the topical application of indomethacin in rat brain did not significantly affect the adenosine-induced vasodilation, whereas it completely blocked the Ang II–induced vasodilation. Therefore, we repeated that the administration of indomethacin blocked the cerebral vasodilation induced by Ang II at 10⁻⁵ mol/L supports the hypothesis that the cerebral arteriolar dilation induced by Ang II may be mediated by prostaglandins through the cyclooxygenase pathway.

Ang-(1-7) has a chemical structure similar to that of Ang II. In our experiment, Ang-(1-7) caused cerebral...
vasodilation similar to that caused by Ang II, and the vasodilation was also blocked by indomethacin. It was also found that Ang-(1-7) could increase PGE$_2$ synthesis in the vasa deferentia with a potency equivalent to that of Ang II. The results suggest that Ang-(1-7)–induced cerebral vasodilation in piglet may be through the same mechanism as Ang II.

A recent in vitro study suggested that Ang II might cause nitric oxide production by neurons. However, if the Ang II– or Ang-(1-7)–induced vasodilation in our experiment was also mainly mediated by nitric oxide, the vasodilation should not be blocked by indomethacin. Thus, it is quite unlikely that Ang II– or Ang-(1-7)–induced vasodilation in piglet was mainly through the nitric oxide pathway.

In summary, Ang-(1-7) and Ang II can cause mild dilation of the cerebral arterioles of piglet. Further, arteriolar dilation appears to be mediated by enhanced production of prostaglandins.

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