Vasodilator Action of Angiotensin-(1-7) on Isolated Rabbit Afferent Arterioles

YiLin Ren, Jeffrey L. Garvin, Oscar A. Carretero

Abstract—Recent studies have shown that angiotensin-(1-7) (Ang-[1-7]), which is generated endogenously from both Ang I and II, is a bioactive component of the renin-angiotensin system and may play an important role in the regulation of blood pressure. However, little is known about its role in regulating the reactivity of the afferent arteriole or the mechanism(s) involved. We hypothesized that Ang-(1-7), acting on specific receptors, participates in the control of afferent arteriole tone. We first examined the direct effect of Ang-(1-7) on rabbit afferent arterioles microperfused in vitro, and we tested whether endothelium-derived relaxing factor/NO and cyclooxygenase products are involved in its actions. To assess the vasodilator effect of Ang-(1-7), afferent arterioles were preconstricted with norepinephrine, and increasing concentrations of Ang-(1-7) were added to the lumen. We found that 10^{-10} to 10^{-6} mol/L Ang-(1-7) produced dose-dependent vasodilatation, increasing luminal diameter from 8.9±1.0 to 16.3±1.1 μm (P<0.006). Indomethacin had no effect on Ang-(1-7)-induced dilatation. N^ \text{6}-\text{nitro-\text{l}-arginine methyl ester, a NO synthesis inhibitor, abolished the dilatation induced by Ang-(1-7). We attempted to determine which angiotensin receptor subtype is involved in this process. We found that 10^{-7} mol/L [d-Ala^7−]−Ang-(1-7), a potent and selective Ang-(1-7) antagonist, abolished the dilatation induced by Ang-(1-7). An angiotensin II type 1 receptor antagonist (L158809) and an angiotensin II type 2 receptor antagonist (PD 123319) at 10^{-7} mol/L had no effect on Ang-(1-7)-induced dilatation. Our results show that Ang-(1-7) causes afferent arteriole dilatation. This effect may be due to production of NO, but not the action of cyclooxygenase products. Ang-(1-7) has a receptor-mediated vasodilator effect on the rabbit afferent arteriole. This effect may be mediated by Ang-(1-7) receptors, because angiotensin type 1 and type 2 receptor antagonists could not block Ang-(1-7)-induced dilatation. Thus, our data suggest that Ang-(1-7) opposes the action of Ang II and plays an important role in the regulation of renal hemodynamics. (Hypertension. 2002;39:799-802.)

Key Words: arterioles ■ angiotensin ■ nitric oxide ■ prostaglandins ■ receptors, angiotensin

Angiotensin II (Ang II) is believed to be the principal bioactive end product of both the circulating system and tissue renin-angiotensin system (RAS). Recent reports have suggested that important central and peripheral actions of the RAS may be conveyed by shorter sequences of Ang peptides, suggested that important central and peripheral actions of the tissue renin-angiotensin system (RAS). Recent reports have

A major target organ of the RAS is the kidney, where Ang II plays a pivotal role in regulation of the renal microcirculation, glomerular filtration, tubular transport, and renal growth.5–6 Enzymes involved in Ang-(1-7) biosynthesis and degradation are abundant in the kidney.7,8 There is growing evidence that just like Ang II, Ang-(1-7) plays a physiological role in the control of water and sodium balance, mainly through its effects on the kidney.9–11 Although the tubular effects of Ang-(1-7) have been studied extensively, its direct effect on the renal vasculature has not been investigated to our knowledge. We hypothesized that Ang-(1-7), acting on specific receptors, participates in the control of afferent arteriole (Af-Art) tone. Our results clearly show that Ang-(1-7) causes Af-Art dilatation. This effect is due to production of NO, but not the actions of cyclooxygenase products. Ang-(1-7) has a novel receptor-mediated dilator effect on the rabbit Af-Art, which appears to be mediated by an angiotensin receptor subtype other than angiotensin II type 1 (AT_1) or type 2 (AT_2).

Methods

We used methods similar to those described previously12,13 to isolate and microperfuse Af-Arts. Briefly, young male New Zealand white rabbits (1.2 to 2.5 kg; Covance, Denver, Pa), fed standard rabbit chow (Ralston Purina) and tap water ad libitum, were anesthetized with ketamine (50 mg/kg IM) and xylazine (10 mg/kg IM) and given an intravenous injection of heparin (500 U). The kidneys were sliced along the corticomedullary axis. Slices were placed in ice-cold

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minimum essential medium (GIBCO) containing 5% BSA (Sigma Chemical) and dissected under a stereomicroscope (SZH, Olympus) as described previously.12,13 A single superficial Af-Art and intact glomerulus were microdissected from each rabbit. Using a micropipette, the arteriole was transferred to a temperature-regulated chamber mounted on an inverted microscope (IMT-2, Olympus) with Hoffman modulation and then cannulated with an array of glass pipettes. Af-Arts were perfused from the proximal end in an orthograde direction. Intraluminal pressure was measured by Landis’ technique, using a fine pipette introduced into the arteriole through the perfusion pipette, and was maintained at 60 mm Hg throughout the experiment.

The arteriole was perfused with oxygenated minimum essential medium containing 5% BSA. The bath, which was exchanged continuously, was identical to the arteriolar perfusate except that it contained 0.15% BSA. Micropipettes and cannulation of the arteriole were completed within 60 minutes at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-minute equilibration period was allowed before any measurements. Images of the arteriole were displayed at magnifications up to 1980X and recorded with a Sony video system consisting of a camera (DXC-755), monitor (H11003), and video recorder (EDV-9500). Diameter was measured with an image-analysis system (Universal Imaging).

**Experimental Protocols**

**Response of Preconstricted Af-Arts to Ang-(1-7)**

We first examined the effect of Ang-(1-7) on Af-Art diameter. After the 30-minute equilibration period, increasing concentrations of Ang-(1-7) (10⁻¹⁰ to 10⁻⁶ mol/L) were added to the lumen; the diameter was monitored for 10 minutes at each dose. In the second series of experiments, to examine the possible vasodilator action of Ang-(1-7), we first constricted Af-Arts by 40% to 50% with noradrenaline and then added increasing concentrations of Ang-(1-7) to the lumen.

**Effect of NO Synthesis Inhibition on Ang-(1-7)-Induced Vasodilatation**

To determine whether NO mediates Ang-(1-7)-induced vasodilatation, we added 10⁻⁷ mol/L N²-nitro-L-arginine methyl ester (L-NAME) to the arteriolar perfusate after the equilibration period to inhibit NO synthesis. Fifteen minutes later, the arteriole was preconstricted with noradrenaline, and the effect of Ang-(1-7) was examined as in protocol 1.

**Effect of Cyclooxygenase Inhibition on Ang-(1-7)-Induced Vasodilatation**

Indomethacin was added to the bath and lumen at a concentration of 5×10⁻³ mol/L from the equilibration period to the end of the experiment. We preconstricted the Af-Art with noradrenaline as described in protocol 1 and examined the effect of intraluminal Ang-(1-7).

**Effect of an AT₁, AT₂, or Ang-(1-7) Receptor Antagonist on Af-Arts**

To determine whether Ang-(1-7)-induced dilatation of preconstricted Af-Arts is mediated by AT₁ or AT₂ or whether it can be blocked by an Ang-(1-7) receptor antagonist, we conducted similar protocols except that an AT₁, AT₂, or Ang-(1-7) antagonist at 10⁻⁶ mol/L was added to the luminal perfusate throughout the experiment.

**Data Analysis**

Values are expressed as mean±SEM. Paired t tests were used to examine whether the diameter at a given concentration differed from the control value within each group. When more than 1 comparison was made, Bonferroni’s multiple comparison adjustment was used. P<0.006 was considered significant.

**Results**

Norepinephrine decreased diameter to 47% of baseline, from 19.0±0.8 to 8.9±0.9 μm (Figure 1). When Ang-(1-7) was added to the lumen, diameter increased in a dose-dependent manner (P<0.006, n=10). Maximal dilatation was obtained at 10⁻⁶ mol/L, which increased diameter to 16.4±1.0 μm (86% of baseline). The ED₅₀ of the Ang-(1-7) dilator response was 2 nmol/L. When the Af-Art was not preconstricted, basal luminal diameter was 17.7±1.6 μm and was not affected by Ang-(1-7) (n=4).

First we investigated the mechanism by which Ang-(1-7) dilates the Af-Art. After L-NAME pretreatment, basal diameter decreased from 22.3±1.34 to 17.8±2.0 μm, and norepinephrine constricted arterioles further (from 17.8±2.0 to 6.6±0.7 μm). In these arterioles, Ang-(1-7) did not produce dilatation (Figure 2). Pretreatment with indomethacin did not alter basal diameter or the vasodilator response of preconstricted Af-Arts to Ang-(1-7). When Ang-(1-7) was added to arterioles preconstricted to 11.5±1.2 μm with norepinephrine, diameter increased to the same extent as the nontreated group; diameter was 12.5±1.4, 13.9±1.3, 15.8±1.2, 16.1±1.2, and 17.2±1.0 μm at 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³, and 10⁻² mol/L, respectively (Figure 2). Maximal dilatation occurred at 10⁻⁶ mol/L, which returned diameter to 83% of baseline.

**Figure 1.** Effect of Ang-(1-7) on Af-Art luminal diameter with (●; n=10) and without norepinephrine preconstriction (○; n=4). *P<0.006 vs pre-Ang-(1-7) values.

**Figure 2.** Effect of Ang-(1-7) on norepinephrine-preconstricted luminal diameter in L-NAME– or indomethacin-treated and non-treated Af-Arts (○; n=10). L-NAME–treated arterioles (●; n=6) did not dilate in response to Ang-(1-7). Indomethacin did not alter the dilator response of preconstricted Af-Arts to Ang-(1-7) (▲; n=10). *P<0.006 vs pre-Ang-(1-7) values.
Next we attempted to determine which angiotensin receptor subtype is involved in this process. Addition of the Ang-(1-7) antagonist [d-Ala7]-Ang-(1-7) did not affect luminal diameter (19.2±1.0 versus 19.8±0.8 μm; n=6). In arterioles preconstricted with norepinephrine from 19.8±0.8 to 9.7±0.8 μm, the Ang-(1-7) antagonist abolished Ang-(1-7)-induced dilation (9.9±1.0, 9.5±0.8, 9.6±1.0, and 9.2±1.0 μm at 10−6, 10−5, 10−4, and 10−3 mol/L Ang-(1-7), respectively) (Figure 3). Antagonists specific for the AT1 or AT2 receptor failed to block the vasodilator action of Ang-(1-7). In the presence of the AT1 antagonist L158809 (Figure 3), when arterioles were preconstricted from 20±1.0 to 8.8±1.9 μm with norepinephrine, Ang-(1-7) induced dilation (13.7±1.8, 16.1±1.3, 17.6±1.3, and 18.3±1.8 μm at 10−6, 10−5, 10−4, and 10−3 mol/L, respectively). Similarly, when Ang-(1-7) was added to arterioles preconstricted to 9.6±1.5 μm in the presence of the AT1 antagonist PD 123319, diameter increased to the same extent as the nontreated Af-Arts (13.4±1.8, 16.1±1.3, 17.4±0.9, and 17.8±1.1 at 10−6, 10−5, 10−4, and 10−3 mol/L, respectively) (Figure 3).

Discussion

We investigated the direct effect of Ang-(1-7) on the Af-Art, as well as the possible pathways involved. We found that in the isolated micropерfused rabbit Af-Art, Ang-(1-7) induces dilation that appears to be receptor-mediated, because it was blocked by a specific Ang-(1-7) antagonist. Moreover, our results suggest that Ang-(1-7)-induced vasodilatation may be caused by production of NO but not by the actions of cyclooxygenase metabolites.

Several studies have shown that Ang-(1-7) produces dose-dependent dilation of coronary artery rings, the aorta, and mesentery artery. The kidney is a critically important target organ for the RAS. The action of Ang-(1-7) in the renal nephron has been studied extensively. Renal infusion of Ang-(1-7) produced marked diuresis and natriuresis in isolated and intact kidneys of Sprague-Dawley and Wistar rats. Small increases in glomerular filtration rate have been observed after Ang-(1-7) infusion in isolated rat kidneys, suggesting that Ang-(1-7) may have a dilatory effect on the renal microcirculation or mesangial cells. We believe our studies provide the first direct evidence that Ang-(1-7) relaxes preconstricted Af-Arts in a dose-dependent manner.

Accumulating evidence suggests that Ang-(1-7) stimulates the synthesis and release of vasodilator prostaglandins, augments the metabolic actions of Bradykinin, and increases the release of NO in other vessels; however, the mechanism by which it dilates renal vessels is unknown. In our study, pretreatment with L-NAME abolished Ang-(1-7)-induced dilatation of preconstricted Af-Arts, suggesting that this process depends on NO synthesis. It was reported that in porcine coronary arteries, Ang-(1-7)-stimulated NO synthesis induced endothelium-dependent relaxation, because relaxation was complete when the endothelium was intact but absent when it was removed. Preincubation of arterial rings with the NO synthase inhibitor L-nitro-arginine abolished the vasodilator response to Ang-(1-7). Similarly, le Tran and Forster reported that Ang-(1-7) caused concentration-dependent relaxation that was more pronounced when the endothelium was intact. Ang-(1-7) has been found to stimulate the release of vasodilator prostaglandins in rabbit aortic smooth muscle cells, and long-term intravenous infusion of Ang-(1-7) in spontaneously hypertensive rats lowered arterial pressure accompanied by significant diuresis and natriuresis and an increase in urinary prostaglandins, raising the possibility that prostaglandins may be involved in Ang-(1-7)-induced renal vasodilatation. However, both le Tran and Forster and Brosnihan et al reported that exposure of the rat aorta or coronary vessels to the cyclooxygenase inhibitor indomethacin had no effect on the relaxation response produced by Ang-(1-7), suggesting that prostaglandins are not involved in its action. Moreover, meclofenamate (a cyclooxygenase inhibitor) did not block the vasodilatation caused by Ang-(1-7). In our study, we found that blocking cyclooxygenase with indomethacin failed to alter the action of Ang-(1-7). In control preparations, Ang-(1-7) increased diameter to 86% of basal values, whereas in indomethacin-treated vessels diameter returned to 83% of baseline. The apparent difference seen in Figure 2 is caused by the difference in basal diameter. Consequently, these studies provide evidence that prostaglandins may not play a major role in the renal vasodilator actions of Ang-(1-7).

There is growing evidence that the actions of Ang-(1-7) in the kidney and other sites are mediated by specific receptors. In the kidney, the antiuretic action of Ang-(1-7) in water-loaded rats was blocked by the selective Ang-(1-7) antagonist A-779, which has a very low affinity for classical Ang II receptor subtypes (AT1 and AT2). Brosnihan et al showed that Ang-(1-7) dilates canine coronary arteries by a non-AT1 or non-AT2 receptor. Scatchard analysis of saturation isotherms of endothelial cells from the bovine thoracic aorta shows that 125I–Ang-(1-7) binds to bovine aortic endothelial cells with an affinity of 19 nmol/L and a density of 1351 fmol/mg protein. In competition studies, specific binding of 125I–Ang-(1-7) was blocked by [Sar1, Ile8]–Ang II and [d-Ala7]–Ang-(1-7). In contrast, neither AT1- nor AT2-selective antagonists significantly competed for 125I–Ang-(1-7)
(1-7) binding.29 These results are in agreement with our finding that Ang-(1-7) has a receptor-mediated dilator effect on the rabbit Af-Art that can be blocked by a selective antagonist, [d-Ala²]-Ang-(1-7), but not by AT₁/AT₂ receptor antagonists. Although further studies are needed to demonstrate the existence of specific Ang-(1-7) receptors, the observations made with the Ang-(1-7) analogue [d-Ala²]-Ang-(1-7) strongly suggest that a receptor other than the AT₁ or AT₂ receptor mediates the biological actions of Ang-(1-7). However, it should be noted that under certain conditions, the effects of Ang-(1-7) may be blocked by losartan or, to a variable extent, by AT₂ receptor antagonists,20,30,31 suggesting a heterogeneity of Ang-(1-7) receptors sensitive to either AT₁ or AT₂ antagonists.

In conclusion, our results suggest that Ang-(1-7) serves as a vasodilator in Af-Arts by stimulating the endothelium to produce NO, which in turn maintains low basolateral AF-Art resistance and counteracts the vasoconstriction induced by Ang II. We have also provided evidence that this effect may be mediated by Ang-(1-7) receptors, because AT₁ and AT₂ receptor antagonists could not block Ang-(1-7)-induced dilatation. This suggests that Ang-(1-7) opposes the action of Ang II and plays an important role in regulation of renal hemodynamics.

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References
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