Angiotensin-(1–7) Reduces Smooth Muscle Growth After Vascular Injury

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Abstract—Regulation of vascular smooth muscle cell growth is critical to the maintenance of normal blood flow and vessel patency. Angiotensin-(1–7) [Ang-(1–7)] inhibits proliferation of vascular smooth muscle cells in vitro and opposes the mitogenic effects of angiotensin II. The present study investigated whether Ang-(1–7) inhibits vascular smooth muscle cell growth in vivo by determining its effect on neointimal formation and medial remodeling in balloon-injured carotid arteries. The carotid arteries of adult male Sprague-Dawley rats were injured with a balloon embolectomy catheter. Ang-(1–7) in saline (24 μg/kg per hour) or saline alone was infused intravenously for 12 days after injury. Pumps containing bromodeoxyuridine were implanted at the same time to determine DNA synthesis. Intravenous infusion increased plasma Ang-(1–7) to 166.0±41.2 fmol/mL (n=6) compared with 46.9±4.2 fmol/mL (n=8) in saline-infused rats. Plasma concentrations of Ang II were not changed by Ang-(1–7) infusion. Elevation in circulating Ang-(1–7) had no effect on either blood pressure or heart rate compared with saline controls. Histomorphometric analysis of carotid arteries indicated that Ang-(1–7) infusion significantly reduced neointimal area compared with rats infused with saline (0.063±0.011 versus 0.100±0.009 mm²; P<0.05). In contrast, Ang-(1–7) infusion had no effect on medial area of the injured or the contralateral uninjured artery compared with saline controls. Ang-(1–7) infusion also reduced the rate of DNA synthesis in both the neointima and the media of the injured vessels. Therefore, exogenous Ang-(1–7) inhibited vascular smooth muscle cell proliferation associated with balloon-catheter injury. Similar increases in endogenous plasma Ang-(1–7) and inhibition of neointimal growth were observed in rats after angiotensin-converting enzyme inhibitor or angiotensin type 1 receptor antagonist administration, suggesting that Ang-(1–7) may contribute to the in vivo antiproliferative effects of these agents on vascular smooth muscle. (Hypertension. 1999;33[part II]:207-211.)

Key Words: angiotensin ■ angiotensin II ■ muscle, smooth ■ vascular injury ■ vascular proliferation ■ hyperplasia

Angiotensin-(1–7) [Ang-(1–7)] is a biologically active member of the renin-angiotensin peptide family (as recently reviewed1). Ang-(1–7) is derived from angiotensin I (Ang I) and angiotensin II (Ang II) by endogenous tissue peptidases2 and is present in tissues and plasma at concentrations similar to Ang II.3–5 Plasma Ang-(1–7) is elevated after treatment of animals or humans with angiotensin-converting enzyme (ACE) inhibitors that reduce Ang II production or angiotensin type I (AT1) receptor antagonists that block Ang II–mediated events.3–5 The first recognized responses to Ang-(1–7) were the stimulation of vasopressin release from neuropeptidergic neurons6 and the release of prostaglandins,7–10 effects also produced by Ang II. However, subsequent studies showed that Ang-(1–7) is devoid of the vasoconstrictor, central pressor, and thirst-stimulating actions of Ang II. In fact, new findings suggest that Ang-(1–7) opposes many of the known actions of Ang II.1

Although Ang II is a potent vasoconstrictor, recent results suggest that Ang-(1–7) regulates arterial pressure by counteacting the pressor effects of Ang II. Ang-(1–7) reduced the blood pressure of spontaneously hypertensive rats11 and renovascular hypertensive dogs,12 while central neutralization of Ang-(1–7) with a selective antibody increased the blood pressure of rats.13 The heptapeptide relaxed canine coronary artery rings through the release of nitric oxide, bradykinin, or both.14 Ang-(1–7) released vasorelaxant prostacyclin from cultured vascular cells,7–9 while indomethacin abolished the vasodilator effects of Ang-(1–7) in pithed rats15 and piglet pial arterioles.16 Recent studies by Iyer et al17,18 in spontaneously hypertensive rats chronically treated with both an ACE inhibitor and an AT1 receptor antagonist showed a rapid pressor response to administration of a monoclonal antibody to Ang-(1–7), a nephrilysin inhibitor, or the nonselective angiotensin peptide antagonist [sarcosine1-threonine8]angiotensin II ([Sar1-Thr8]Ang II). Because these agents effectively reduced circulating Ang-(1–7) or inhibited its activity, the endogenous depressor action of Ang-(1–7) was unmasked. Taken together, these results suggest that Ang-(1–7) opposes the pressor effects of Ang II.

Ang-(1–7) also opposes the mitogenic response to Ang II in cultured vascular smooth muscle cells (VSMC). We showed that Ang-(1–7) inhibited the serum-, Ang II–, or neointimal arterial injury model in vivo.19,20 Ang-(1–7) reduced the rate of DNA synthesis in both the neointima and the media of the injured vessels. Therefore, exogenous Ang-(1–7) inhibited vascular smooth muscle cell proliferation associated with balloon-catheter injury. Similar increases in endogenous plasma Ang-(1–7) and inhibition of neointimal growth were observed in rats after angiotensin-converting enzyme inhibitor or angiotensin type 1 receptor antagonist administration, suggesting that Ang-(1–7) may contribute to the in vivo antiproliferative effects of these agents on vascular smooth muscle. (Hypertension. 1999;33[part II]:207-211.)

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platelet-derived growth factor–stimulated growth of cultured VSMC,19 in contrast to the growth-stimulatory effects of Ang II.20,21 Ang-(1–7) caused a dose-dependent inhibition of [3H]thymidine incorporation into serum-stimulated VSMC as well as a decrease in cell number. Therefore, we examined the effect of exogenous Ang-(1–7) on neointimal formation and medial remodeling in balloon-injured carotid arteries to determine whether Ang-(1–7) also attenuated stimulated vascular growth in vivo.

Methods

Animals

Twelve- to 14-week-old male Sprague-Dawley rats (body weight, 300 to 400 g), derived from founder animals acquired from the Zentralinstitut für Versuchstierkunde, Hannover, Germany, were bred and raised at the American Association for Accreditation of Laboratory Animal Care–accredited animal facility of Wake Forest University School of Medicine. All animals were provided normal rat chow (Prolab R-M-L 3000 containing 0.44% sodium, Purina Mills, Inc) and tap water ad libitum and were housed in individual cages with a 12-hour dark/light cycle.

Experimental Protocol

Rats were randomly divided into 2 groups of 6 to 8 rats each. Each rat was anesthetized with an intramuscular injection of ketamine hydrochloride (40 mg/kg; Ketaset, Fort Dodge Laboratories, Inc), xylazine (4 mg/kg; Rompun, Miles), and acepromazine maleate (0.5 mg/kg; Promazine, Fort Dodge Laboratories, Inc). An osmotic minipump (model 2ML2, Alzet osmotic pump, Alza) implanted subcutaneously delivered via catheter either 24 μg/kg per hour of Ang-(1–7) (Bachem) in saline or saline alone (5 μL/h) into the jugular vein. A second subcutaneous minipump delivered the non-radioactive thymidine analogue 5-bromo-2'-deoxyuridine (BrdU) (0.8 mg/kg per day in saline, 5 μL/h; Boehringer Mannheim).

In anesthetized animals implanted with osmotic pumps for drug administration, the left common carotid artery wall was injured with an embolectomy balloon catheter (2F Fogarty, Baxter Healthcare) to induce neointimal formation, as described by Clowes et al.22 The left and right carotid bifurcations and external carotid branches were exposed by an incision through the skin of the ventral neck. After ligation of the external carotid artery, the embolectomy catheter was introduced into the left common carotid artery and passed to the aorta. Endothelial denudation and artery wall injury were achieved by simultaneously withdrawing and twisting the catheter through the common carotid while the catheter balloon was inflated with 0.1 mL 0.9% NaCl. This process was repeated 3 times before removal of the catheter and ligation of the external carotid artery. The right carotid bifurcation was similarly exposed as a sham-operated control, and the right external carotid artery was ligated before wound closure.

After 12 days of peptide infusion, the rats were anesthetized with the combination of ketamine, xylazine, and acepromazine. Blood pressure and heart rate were measured on a multichannel polygraph (model 2000, Gould) by insertion of an 18-gauge angiocatheter (Baxter Healthcare) into the abdominal aorta and attachment of the catheter to a strain gauge transducer (Spectramed). Blood (7 mL) was withdrawn for angiotensin peptide measurement, as described below. A 5% solution of Evans blue dye (0.3 mL) was administered via the catheter to identify denuded and injured artery, followed by a bolus injection of pentobarbital sodium (25 mg/kg). The arteries were perfusion-fixed in situ through the arterial and ligation of the external carotid artery. The right carotid bifurcation was similarly exposed as a sham-operated control, and the right external carotid artery was ligated before wound closure.

Effect of Intravenous Infusion of Ang-(1–7) on Blood Pressure, Heart Rate, and Angiotensin Peptides

Infusion of Ang-(1–7) had no effect on blood pressure or heart rate of Sprague-Dawley rats treated with the peptide. In Ang-(1–7)-treated rats, the diastolic pressures (45±9 mm Hg; n=5), systolic pressures (105±22 mm Hg; n=5), and heart rates (351±18 bpm; n=5) were unchanged compared with those of saline-infused rats (52±11 mm Hg, 97±14 mm Hg, and 382±16 bpm, respectively; n=8). Plasma concentrations of Ang-(1–7) and Ang II were measured by radioimmunoassay. Figure 1 illustrates that 12 days of intravenous infusion of Ang-(1–7) (24 μg/kg per hour) caused a 3.5-fold increase in the circulating level of the peptide, from 46.9±4.2 fmol/mL in saline-infused rats (n=8) to 166.0±41.2 fmol/mL in saline-infused rats (n=8).
in peptide-infused animals (n=6; P<0.05). Plasma Ang II was not affected by the infusion of Ang-(1–7).

**Effect of Intravenous Infusion of Ang-(1–7) on Neointimal Formation**

Balloon-catheter injury of carotid arteries caused the formation of a neointima in both saline- and Ang-(1–7)–treated rats. Contralateral uninjured carotid arteries had no discernible intima. Intravenous infusion of Ang-(1–7) significantly reduced the cross-sectional area of the neointima, as shown in Figures 2 and 3. Ang-(1–7) caused a 37% reduction in the cross-sectional area of the neointima, from 0.100±0.009 mm² in saline-infused rats (n=8) to 0.063±0.011 mm² in Ang-(1–7)–infused rats (n=6; P<0.05). In contrast, Ang-(1–7) infusion had no effect on the medial area of injured or uninjured carotid arteries compared with saline-infused rats. Correspondingly, the neointima/media ratio was reduced in Ang-(1–7)–infused rats compared with saline-infused rats (from 0.62±0.03 in saline-infused rats to 0.42±0.07 in Ang-(1–7)–infused animals).

**Effect of Intravenous Infusion of Ang-(1–7) on DNA Synthesis in the Neointima and Media**

The rate of DNA synthesis was measured by the incorporation of BrdU into newly synthesized DNA in the neointima and the media of injured arteries and the media of uninjured carotid arteries. The rate of DNA synthesis was defined as the cumulative BrdU labeling index, calculated by the percentage of nuclei within the neointima or media incorporating BrdU during the 12-day infusion period. BrdU was incorporated into a significant number of cell nuclei in both the neointima (92.5±0.4%) and the media (48.1±2.4%) of the injured carotid arteries of saline-infused rats. Ang-(1–7) infusion significantly (P<0.05) reduced the rate of DNA synthesis in both the neointima and the media of the injured arteries, as shown in Figure 4. DNA synthesis was rare or absent in the media of the uninjured carotid arteries of saline- or Ang-(1–7)–infused rats (data not shown).

**Discussion**

Infusion of Ang-(1–7) into normotensive Sprague-Dawley rats inhibited VSMC proliferation in vivo after vascular injury. This study extends our previous work showing that Ang-(1–7) reduced both the amount of mitogen-stimulated [³H]thymidine incorporation and cell number in cultured VSMC treated in vitro with Ang-(1–7). DNA synthesis in both the neointima and the media of the injured artery and neointimal area were significantly reduced by Ang-(1–7) infusion compared with saline-infused rats. The increase in plasma Ang-(1–7) had no effect on blood pressure or heart rate, indicating that the antiproliferative effect of the peptide was not due to alterations in hemodynamic forces.

Ang-(1–7) inhibited the serum-, platelet-derived growth factor–, and Ang II–stimulated increase in [³H]thymidine incorporation in cultured VSMC. Infusion of Ang II into Sprague-Dawley rats stimulates vascular smooth muscle growth in conjunction with increased blood pressure. Vascular growth in response to coinfusion of Ang II and hydralazine demonstrated that the Ang II–mediated increase
in proliferation was independent of its pressor effect. 21 This is in agreement with a reduction in neointimal formation after vascular injury by treatment with either ACE inhibitors or AT1 receptor antagonists. 23–25 ACE inhibitors increase circulating kinins while decreasing or having no effect on plasma Ang II, suggesting that bradykinin participates in the antiproliferative effects of ACE inhibition. Farhy et al 26 showed that a B2 antagonist attenuated the ACE inhibitor–reduced neointimal formation through blockade of kinin-stimulated migration of medial cells. 27 However, circulating levels of Ang-(1–7) increase 25–50-fold during ACE inhibition, 3–5 secondary to increased Ang I conversion to Ang-(1–7) and inhibition of Ang-(1–7) breakdown by ACE. 28 Since Ang-(1–7) inhibits vascular proliferation in vitro and in vivo, the heptapeptide may participate in the antiproliferative effects of ACE inhibition.

Ang-(1–7) reduced the cross-sectional area of the neointima and decreased the rate of DNA synthesis. In contrast, the heptapeptide decreased the rate of DNA synthesis in the media of the injured artery but did not reduce the medial cross-sectional area. The response to vascular injury in the rat carotid artery follows a pattern of medial smooth muscle proliferation, migration of smooth muscle cells across the internal elastic lamina to form the neointima, and neointimal smooth muscle proliferation. 22,29 Inhibition of VSMC migration by Ang-(1–7) could account for the decreased rate of medial DNA synthesis without a significant decrease in medial area. Alternatively, Ang-(1–7) may attenuate medial VSMC apoptosis to oppose the decreased rate of medial DNA synthesis. deBlois et al 30 showed that treatment of spontaneously hypertensive rats with ACE inhibitors altered vascular cell apoptosis in addition to inhibiting vascular growth.

The angiotensin receptor coupled to the inhibition of VSMC growth in vivo was not investigated in the present study. However, in cultured VSMC, we showed that inhibition of serum-stimulated [3H]thymidine incorporation by Ang-(1–7) was prevented by the sarcosine analogues of Ang II—[Sar1-Thr8]-Ang II or [sarcosine-isoleucine] angiotensin II ([Sar1-Ile8]-Ang II)—but not by AT1 or AT2 receptor antagonists. 19 We identified a novel non-AT1, non-AT2 Ang-(1–7) receptor in bovine aortic endothelial cells 31 and canine coronary arteries, 1 suggesting that Ang-(1–7) activates a unique angiotensin peptide receptor. The Ang-(1–7)–mediated relaxation of preconstricted canine coronary arteries was prevented by sarcosine analogues of Ang II but not by AT1 or AT2 receptor antagonists. 14 In addition, Iyer et al 17,18 recently showed that the antihypertensive effect of combined ACE inhibition and AT1 receptor antagonism was attenuated by an antibody to Ang-(1–7), an inhibitor of Ang-(1–7) formation or [Sar1-Thr8]-Ang II but not by an AT2 receptor antagonist. Collectively, these results suggest that Ang-(1–7) activates a novel Ang-(1–7) receptor to regulate blood pressure and inhibit VSMC proliferation.

Although we did not investigate the mechanisms by which Ang-(1–7) inhibits vascular proliferation, we previously showed that Ang-(1–7) released prostacyclin from cultured VSMC. 7–9 Blockade of prostaglandin production by indomethacin attenuates the inhibition of VSMC thymidine incorporation by Ang-(1–7) (E.A. Tallant, unpublished data, 1998). Prostacyclin inhibited the growth of cultured VSMC. 32 In addition, overexpression of prostacyclin synthase in rat VSMC increased prostacyclin production and decreased DNA synthesis in response to serum stimulation, providing further proof that prostacyclin inhibits VSMC growth. 33 Treatment with a stable prostacyclin analogue also reduced neointimal growth in vivo after vascular injury to the rabbit aorta. 34 Although other mechanisms may participate in the Ang-(1–7)–mediated inhibition of vascular growth (such as production of the growth inhibitor nitric oxide), our results suggest that Ang-(1–7) may inhibit vascular growth by the production of prostacyclin.

VSMC growth is regulated by hemodynamic, blood-borne, and tissue-derived signals to maintain normal blood flow and vessel patency. 22,29 Proliferative and antiproliferative factors tend to balance VSMC growth. Artery wall thickness is maintained in normal adult arteries by permitting only a slow rate of smooth muscle cell proliferation to adjust compliance. Abnormal vascular growth is an important etiologic factor in vessel patency. 22,29 Proliferative and antiproliferative factors tend to balance VSMC growth. Artery wall thickness is maintained in normal adult arteries by permitting only a slow rate of smooth muscle cell proliferation to adjust compliance. Abnormal vascular growth is an important etiologic factor in the pathology of many vascular disorders, including hypertension. The results of the present study demonstrate that infusion of exogenous Ang-(1–7) results in a reduction in neointimal formation, suggesting that Ang-(1–7) may inhibit VSMC growth in vivo. Since plasma Ang-(1–7) is elevated after treatment with ACE inhibitors, our results suggest that
Ang-(1–7) may contribute to the antiproliferative effects of ACE inhibition.

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