Divergent Roles of Angiotensin II AT₁ and AT₂ Receptors in Modulating Coronary Microvascular Function

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Abstract—Angiotensin II (Ang II) is a potent vasoconstrictor in the peripheral circulation and has been implicated in many cardiovascular diseases associated with elevated oxidative stress. However, its direct vasomotor action and its linkage to oxidative stress–induced vascular dysfunction in the coronary microcirculation remain elusive. In this study, we directly assessed the vasomotor action of Ang II in isolated porcine coronary arterioles and also examined whether Ang II can modulate endothelium-dependent nitric oxide (NO)–mediated dilation via superoxide production. Ang II evoked vasoconstriction at a low concentration (1 nmol/L) and dilations at higher concentrations (>10 nmol/L). Ang II type 1 (AT₁) receptor antagonist losartan abolished vasoconstriction, whereas Ang II type 2 (AT₂) receptor antagonist PD 123319 eliminated vasodilation. Adenosine stimulated a significant arteriolar NO production and dilation. NO synthase inhibitor N⁶-monomethyl-L-arginine (L-NMMA) abolished stimulated NO production and attenuated vasodilation. Pretreating vessels with a subvasomotor concentration of Ang II (0.1 nmol/L, 60 minutes) mimicked inhibitory effects of L-NMMA. Ang II–mediated inhibition was not observed in the presence of L-NMMA or after endothelial removal but was prevented by losartan, superoxide scavenger TEMPOL, or NADPH oxidase inhibitor apocynin. Dihydroethidium staining showed that Ang II elicited losartan- and TEMPOL-sensitive superoxide production in arterioles. These results demonstrate that Ang II evokes AT₁ receptor–mediated vasoconstriction and AT₂ receptor–mediated vasodilation of coronary arterioles. Ang II at a subvasomotor level impairs endothelium-dependent NO-mediated vasodilation of coronary arterioles. Ang II may partly explain the impaired coronary flow regulation in heart diseases associated with an upregulated renin-angiotensin system. (Circ Res. 2003;92:1233–1240.)

Key Words: angiotensin  free radicals  microcirculation  nitric oxide  vasodilation

Angiotensin II (Ang II), the main biological active peptide of the renin-angiotensin system (RAS), plays an important role in cardiovascular homeostasis by contributing to the regulation of blood volume, blood pressure, and peripheral vascular tone. The vasomotor activity of Ang II has been demonstrated in various vascular beds, including renal, cerebral, skeletal muscle, and mesenteric, by acting on either Ang II type 1 (AT₁) or type 2 (AT₂) receptors. However, the action of Ang II in the coronary circulation of an intact heart is controversial. For example, a decrease, an increase, or a transient decrease followed by an increase in coronary flow by Ang II has been reported. Although this inconsistency may be a result of using different animal models or experimental approaches, the complexity of flow regulation in the intact heart may be largely responsible for these diverse findings. For example, it is well accepted that coronary vasomotor tone can be influenced by the neural activity and by the changes in local hemodynamics and cardiac metabolism. Therefore, the precise action of Ang II in the coronary vasculature is difficult to assess in the intact heart, because this peptide has direct and indirect actions on these biological factors. Furthermore, the specific receptor subtypes responsible for the vasomotor action of Ang II in the coronary circulation remain undetermined. Because coronary microvascular tone is the predominant determinant of coronary resistance and thus of coronary blood flow, it is imperative to unequivocally understand the direct effect of Ang II and its cognate receptors on coronary microvessels.

In addition to its direct vasomotor action, Ang II has been recently shown to increase superoxide production in cultured endothelial cells. Therefore, it is likely that Ang II may also modulate vascular tone by generating reactive oxygen species. For example, the released superoxide may influence vasomotor function by impairing nitric oxide (NO)–mediated vasodilation as an NO scavenger. This hypothesis is seemingly supported by a recent observation that long-term (3-day) administration of subpressor levels of Ang II impairs endothelium-dependent aortic relaxation to acetylcholine.
However, it remains unclear whether coronary microvascular function is susceptible to Ang II exposure. Because acute myocardial ischemia (<60 minutes) upregulates cardiac RAS and impairs coronary flow regulation, understanding the vasomotor function and its modulation by a short-term Ang II exposure in the coronary microcirculation is clinically important. Therefore, the goals of the present study were to determine the vasomotor action of Ang II in coronary arterioles and to elucidate whether Ang II can exert a detrimental role in NO-mediated vasodilation through superoxide production in these microvessels.

Materials and Methods

Functional Assessment of Isolated Coronary Arterioles

To determine the response of coronary arterioles to Ang II, pigs (12 to 15 kg) were anesthetized with pentobarbital (20 mg/kg) and ventilated; then the heart was removed and the individual coronary arterioles (60 to 110 μm in internal diameter in situ) were dissected out for in vitro study as previously described. Vessels were cannulated with glass micropipettes and pressurized to 60 cm H₂O intraluminal pressure. The cannulated vessel was bathed in physiologic salt solution (PSS) containing BSA (1%; Amersham) at 37°C. After developing stable basal tone, the concentration-diameter relationships for Ang II (0.1 nmol/L to 1 mol/L) were established. The role of specific Ang II receptors in the Ang II–induced vasomotor responses was assessed in the presence of AT₁ receptor antagonist losartan (1 μmol/L, 30-minute incubation; gift from Merck, West Point, Pa) or AT₂ receptor antagonist PD 123319 (50 μmol/L, 30-minute incubation).

To determine whether Ang II can influence coronary arteriolar dilatations mediated by different signaling mechanisms, isolated coronary arterioles were subjected to various agonist stimulations before and after treating vessels with a subvasomotor concentration (0.1 nmol/L) of Ang II for 60 minutes. Adenosine was used as an activator of NO-mediated vasodilation, and bradykinin was used to probe the prostanoids pathways. 14 The ATP-sensitive potassium (KATP) channel activator pinacidil 12 was used as an endothelium-derived hyperpolarizing factor. 14 The vasodilation elicited by arachidonic acid was used to probe the prostanooids pathways. 14 The ATP-sensitive potassium (KATP) channel activator pinacidil 12 was used as an endothelium-independent agonist to assess the vasodilatory function in response to KATP channel activation. To determine the role of Ang II receptors and superoxide anions in mediating the effect of Ang II, the above vasodilatory functions were examined in the presence of specific Ang II receptor antagonists (1 μmol/L losartan or 50 μmol/L PD 123319) and a membrane-permeable superoxide dismutase mimetic, TEMPOL (1 mmol/L, 60-minute incubation). 15 respectively. The contribution of NADPH oxidase in generating superoxide in the presence of Ang II was assessed by treating vessels with an NADPH oxidase inhibitor, apocynin (100 μmol/L). 16 The role of endothelium in mediating Ang II effect was determined in the blood vessels after endothelial removal by perfusion of a nonionic detergent, CHAPS (0.4%), to the lumen of the vessels as previously described. 17 After a 30-minute equilibration period in PSS at 37°C, vessels were incubated with adenosine (1 μmol/L) or adenosine (1 μmol/L) plus Ang II (0.1 nmol/L) for 60 minutes. In some experiments, losartan (1 μmol/L) or TEMPOL (1 mmol/L) was added to the Ang II solution to study the role of AT₁ receptors and superoxide in NO production. Protein levels in each sample were quantified by bicinchoninic acid protein assay (Pierce) and were used to normalize the NO production.

Measurement of Superoxide

The production of superoxide was evaluated in coronary arterioles with the oxidative fluororescent dye dihydroethidium (DHE). 18 Isolated coronary arterioles (100 to 150 μm in diameter and 3 to 4 mm in length) were incubated in PSS containing vehicle, losartan (1 μmol/L), TEMPOL (1 mmol/L), Ang II (0.1 nmol/L), Ang II and losartan, or Ang II and TEMPOL at 37°C for 60 minutes followed by embedding the arterioles in OCT compound (Tissue-Tek) for cryostat sections. The embedded arterioles were cut into sections 12 μm thick and placed on glass slides. DHE (4 μmol/L) was applied to all tissue sections on the slide, which were incubated in a light-protected humidified chamber at 37°C for 30 minutes and then sealed with coverslips. Images were obtained using a laser scanning confocal microscope (Ulma-l-2, 312, Meridian Instruments). Fluorescence was detected with a 605-nm long-pass filter. Control and experimental tissue sections were placed on the same slide and processed under the same conditions. Laser settings for image acquisition were identical for both control and experimental tissues.

Data Analysis

All data are presented as mean±SE. Statistical comparisons of vasomotor responses and NO production under various treatments were performed with ANOVA when appropriate and tested with Bonferroni multiple-range test. Difference in expression of Ang II receptor subtypes was compared by paired Student’s t test. Significance was accepted at P<0.05.

An expanded Materials and Methods section is available online at http://www.circresaha.org in the data supplement.

Results

Vasomotor Function and Ang II Receptor Activation

The isolated coronary arterioles developed basal tone as 65±3% of maximal diameter (119±7 μm; range, 80 to 160 μm) at 60 cm H₂O intraluminal pressure. The level of basal tone was similar in all treatment groups delineated below. The vasomotor response of arterioles to Ang II (0.1 nmol/L to 1 μmol/L) is presented in Figure 1A. Ang II did not exert vasomotor activity at the lowest concentration (0.1 nmol/L) but caused vasoconstriction at 1 nmol/L. In contrast, coronary arterioles dilated in response to higher concentrations (10 nmol/L to 1 μmol/L). In the presence of losartan, basal tone was not altered, but the vasoconstrictive effect of Ang II at 1 nmol/L was reversed to vasodilation. Furthermore, the vasodilation in response to 10 nmol/L Ang II was significantly

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was isolated from porcine coronary arterioles (3 to 4 vessels, ~100 μm in diameter and 2 to 3 mm in length) and kidney tissue (positive control) based on protocols previously described. 13 Sets of primers specific for AT₁ and AT₂ receptor genes and glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) gene were engineered (Sigma-Genosys). Equal amounts (0.1 μg) of total RNA for each sample were used in RT-PCR as previously delineated. 13 PCR-amplified products were electrophoresed on 1.8% agarose gels and visualized with ethidium bromide staining. The level of expression of Ang II receptor transcripts was normalized to that of GAPDH transcripts.

Measurement of NO Production

The production of NO in isolated coronary arterioles (5 to 7 vessels/sample, ~100 μm in diameter and 1 to 2 mm in length) was evaluated by measuring nitrite using a chemiluminescence NO analyzer (Siever Instruments) as previously described. 11 After a 30-minute equilibration period in PSS at 37°C, vessels were incubated with adenosine (1 μmol/L) or adenosine (1 μmol/L) plus Ang II (0.1 nmol/L) for 60 minutes. In some experiments, losartan (1 μmol/L) or TEMPOL (1 mmol/L) was added to the Ang II solution to study the role of AT₁ receptors and superoxide in NO production. Protein levels in each sample were quantified by bicinchoninic acid protein assay (Pierce) and were used to normalize the NO production.
enhanced (Figure 1A). However, AT2 receptor antagonist PD 123319 significantly enhanced vasoconstriction to Ang II, but the vasodilatory effect was abolished (Figure 1A). These vessels dilated equally to a maximal concentration of sodium nitroprusside (100 μmol/L), indicating that the Ang II receptor antagonists did not exert a nonspecific effect on vasomotor reactivity and that the vessels exhibited similar levels of vasodilatory capacity (Figure 1A). RT-PCR analysis demonstrated that both AT1 and AT2 receptor mRNAs were detected in isolated coronary arterioles (Figure 1B). Normalization of arteriolar Ang II receptor transcripts with GAPDH transcripts (which exhibited a similar level of expression for all samples evaluated) showed that the expression of AT2 was 2-fold greater than that of AT1. In contrast, the expression of AT1 was ~3-fold greater than that of AT2 in the kidney tissue (Figure 1B).

**Effect of Ang II on NO-Mediated Vasodilation to Adenosine**

We have previously demonstrated that coronary arteriolar dilation to adenosine is partly mediated by NO released from the endothelium. To investigate whether Ang II can modulate NO-mediated vasodilation, the coronary arteriolar response to adenosine was examined before and after incubation with a subvasomotor concentration of Ang II (0.1 μmol/L) for 60 minutes. In the absence of Ang II, adenosine evoked dilation of coronary arterioles in a concentration-dependent manner (Figures 2A and 2B). This vasodilation was attenuated in an identical manner by either denudation (Figure 2A) or treating the vessels with NO synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA) (10 μmol/L; Figure 2B). In the presence of Ang II, basal vascular tone was not altered (before Ang II, 62±1% of maximal diameter; after Ang II, 64±2% of maximal diameter), but the vasodilation to adenosine was significantly attenuated (Figures 2A and 2B). The inhibitory action of Ang II was identical to that produced by endothelial removal (Figure 2A) or by L-NMMA (Figure 2B). Endothelial denudation was verified by complete block of bradykinin-induced dilation (control, 85±6% dilation versus denudation, 4±2% dilation). Vascular smooth muscle function remained intact in these denuded vessels because vasodilation to sodium nitroprusside (1 nmol/L to 10 μmol/L) was not altered (data not shown). However, Ang II failed to exert its inhibitory effect on the adenosine response in denuded (Figure 2A) or L-NMMA–treated (Figure 2B) vessels.

**Role of Ang II Receptors and Superoxide in Ang II–Induced Vascular Dysfunction**

In another group of vessels, the involvement of specific Ang II receptors and superoxide in the Ang II–mediated inhibition
of adenosine response was examined. Subsequent incubation of Ang II–treated vessels with losartan, but not PD 123319, normalized the vasodilatory response to adenosine (Figure 3). Similar to losartan treatment, administration of either superoxide scavenger TEMPOL or NADPH oxidase inhibitor apocynin restored the normal vasodilation (Figure 4A). To additionally support the hypothesis that superoxide anions exert a detrimental effect on NO-mediated vasodilation, we examined whether a superoxide generator pyrogallol can alter the adenosine response in coronary arterioles. After a 60-minute incubation with pyrogallol (100 μmol/L), the adenosine-induced vasodilation was significantly attenuated (Figure 4B). In the same vessels, subsequent administration of TEMPOL restored normal vasodilation (Figure 4B).

**Effect of Ang II on Vasodilations to Bradykinin, Arachidonic Acid, and Pinacidil**

Pretreating the vessels with Ang II (0.1 nmol/L) for 60 minutes did not affect the endothelium-dependent vasodilation to a cytochrome P-450 monoxygenase activator bradykinin (Figure 5A). Activation of cyclooxygenase pathway by arachidonic acid (10 μmol/L) caused a 73±2% dilation of coronary arterioles; this dilation was not altered in the presence of Ang II (ie, 73±4% dilation, n=3, data not shown). Furthermore, Ang II had no effect on the vasodilation elicited by smooth muscle K<sub>ATP</sub> channel opener pinacidil (Figure 5B).

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Ang II inhibits coronary arteriolar dilations to adenosine. Vasodilation of isolated coronary arterioles to adenosine was examined before and after (A) endothelial denudation (n=6), incubation with L-NMMA (10 μmol/L, n=6) (B), or pretreatment with a subvasomotor concentration of Ang II (0.1 nmol/L, n=6) (A and B) for 60 minutes. n indicates number of vessels. *P<0.05 vs control.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Blockade of AT<sub>1</sub> receptors prevents Ang II–induced reduction of coronary arteriolar dilation to adenosine. Vasodilation of isolated coronary arterioles to adenosine was examined before and after pretreatment with a subvasomotor concentration of Ang II (0.1 nmol/L) in the absence (n=5) and presence of losartan (1 μmol/L, n=4) or PD 123319 (50 μmol/L, n=5). *P<0.05 vs control.
Effect of Ang II on Adenosine-Induced NO Production

To support the functional study, coronary arteriolar production of NO in response to adenosine in the absence and presence of Ang II (0.1 nmol/L, 60-minute incubation) was measured. Under resting conditions, only a small amount of NO was produced from coronary arterioles (≈69 ± 12 nmol nitrite/g protein, Figure 6). Adenosine (1 μmol/L) stimulated a 3-fold increase in the production of NO from control vessels. However, this stimulated NO production was abol-

Figure 4. Blockade of superoxide production or NADPH oxidase activation prevents Ang II–induced reduction of coronary arteriolar dilation to adenosine. A, Vasodilation of coronary arterioles to adenosine was examined before and after pretreatment with a subvasomotor concentration of Ang II (0.1 nmol/L, n = 9) in the absence and presence of superoxide anion scavenger TEMPOL (1 mmol/L, n = 5) or NADPH oxidase inhibitor apocynin (100 μmol/L, n = 4). B, In another group of vessels, adenosine-induced dilation was examined before and after incubation with superoxide generator pyrogallol (100 μmol/L, n = 7) in the absence and presence of TEMPOL (1 mmol/L, n = 4). n indicates number of vessels. *P < 0.05 vs control.

Figure 5. Pretreatment with a subvasomotor concentration of Ang II (0.1 nmol/L) does not affect coronary arteriolar dilations to bradykinin (n = 5) (A) and pinacidil (n = 7) (B). n indicates number of vessels.
ished in the presence of Ang II. Simultaneous incubation of vessels with Ang II and either losartan (1 \(\mu\)mol/L) or TEMPOL (1 mmol/L) prevented the inhibitory effect of Ang II (Figure 6).

**Ang II–Induced Superoxide Production in Coronary Arterioles**

In the absence of Ang II (ie, vehicle), DHE oxidative fluorescence revealed sparse levels of superoxide throughout the vessel wall (Figure 7). On the other hand, incubation of arterioles with Ang II (0.1 nmol/L, 60 minutes) caused a pronounced increase in fluorescent signals in both endothelial and smooth muscle layers, indicating augmented levels of superoxide in these cell types. The endothelial and smooth muscle layers were identified by setting the scanning threshold to obtain a clear background image of the blood vessel. Neither losartan nor TEMPOL alone significantly affected baseline levels of arteriolar fluorescence, but these two agents markedly reduced the Ang II–induced fluorescent signals for superoxide in both cell types (Figure 7).

**Discussion**

The present study demonstrates that Ang II is a vasoactive agent that causes vasoconstriction or vasodilation, depending on the concentration, in the coronary arterioles. It seems that activation of AT1 receptors by a low concentration of Ang II (1 nmol/L) causes vasoconstriction. In contrast, higher concentrations of Ang II (>1 nmol/L) evoke vasodilation by activating AT2 receptors. Interestingly, a subvasomotor concentration of Ang II elicits superoxide production in coronary arterioles and also selectively inhibits NO production and endothelium-dependent NO-mediated dilation in response to adenosine. The inhibitory effects were prevented by a superoxide scavenger, an NADPH oxidase inhibitor, or an AT1 receptor antagonist, indicating that the activation of AT1 receptors and subsequent generation of superoxide via NADPH oxidase are involved in the detrimental effect of Ang II.

**Ang II and Vascular Tone in Coronary Microvessels**

Ang II has been shown to exert vasomotor activity in various microvascular beds. In the coronary circulation, it has been
reported that Ang II modestly constricts large conduit vessels.19 However, the research on the direct effect of Ang II on coronary microcirculation is sparse. In the intact rabbit heart perfused with constant flow, coronary infusion of Ang II produced a transient increase in coronary pressure followed by a prolonged pressure reduction, suggesting that Ang II may exhibit a complex vasomotor reaction in the coronary microcirculation by producing vasoconstriction followed by vasodilation.8 Because Ang II can modulate cardiac function and metabolism, the direct microvascular action of Ang II is difficult to unequivocally assess in the intact heart. Furthermore, the secondary effects attributable to subsequent flow and pressure changes induced by initial vasomotor reaction to Ang II make the data interpretation more difficult. With these considerations in mind, isolated vessel approaches would be the direct method to address the vasomotor activity of Ang II. To the best of our knowledge, there has only been one isolated vessel study performed in the coronary microvessels.19 With the aid of endothelin-induced tone, this study documented that Ang II caused concentration-dependent dilation of canine coronary arterioles but did not elicit any vasomotor response in porcine coronary arterioles (50 to 150 μm).19 In contrast, we found that Ang II evoked vasoconstriction at a low concentration (1 nmol/L) and vasodilations at higher concentrations (10 nmol/L to 1 μmol/L). The reason for the absence of vasoconstriction to Ang II in the previous study19 is unclear but may be related to the use of pharmacological preconstrictor, because administration of a vasoconstrictor to the skeletal muscle microcirculation was found to abolish Ang II–induced vasoconstriction.20 This is confirmed by our preliminary studies indicating that preconstriction of coronary arterioles with endothelin (1 nmol/L), mimicking the earlier in vitro study in coronary arterioles,19 not only abolished the vasoconstrictor effect of Ang II but also attenuated the vasodilation (n = 3, data not shown). It seems that the reaction of coronary arterioles to Ang II is sensitive to the addition of other vasoconstrictors, which thus masks the normal vascular response to Ang II.

It is generally considered that the direct vasomotor action of Ang II is mediated by at least two Ang II receptor subtypes, AT1 and AT2. An in vivo study indicates that activation of AT1 receptors mediates the vasoconstriction of conduit coronary arteries.21 Although the physiological role of AT2 receptors in the regulation of vascular tone remains controversial, several pharmacological studies implicate that these receptors may mediate vasodilation of coronary arteries.22,23 In contrast to the wealth of information from large conduit vessels, the expression of specific Ang II receptor subtypes in the coronary microcirculation has not been explored despite the fact that receptor binding for Ang II has been identified in coronary microvessels.19 In the present study, we demonstrated that both AT1 and AT2 receptor mRNAs are expressed in the coronary arterioles and that these receptors play a distinct role in vasomotor action, i.e., AT1 receptor–mediated constriction and AT2 receptor–mediated vasodilation. Semiquantitative analysis of the arteriolar Ang II receptor genes showed that the expression of AT2 receptor transcripts was 2-fold greater than that of AT1, which is consistent with the predominance of AT2-mediated vasodilation observed in the functional study. It is interesting to note that we observed a contrasting level of expression in the kidney tissue with a prevalence of AT1 versus AT2. These molecular data seem to reflect previous functional studies showing diverse vasomotor activity in response to Ang II in different vascular beds.1 Moreover, because losartan did not significantly alter the vasodilatory response to high concentrations of Ang II, it seems that the role of AT1 is gradually diminished with increasing Ang II concentration (eg, 0.1 to 1 μmol/L). This is consistent with the findings in the intact heart that the magnitude of coronary vasodilation is increased with increasing Ang II concentrations.4 It should be noted that the receptor antagonists used in the present study did not affect vasodilations to pinacidil and sodium nitroprusside (data not shown), indicating that the inhibitory action of these blockers was not a result of nonspecific suppression of vascular function.

**Ang II and Endothelium-Dependent Dilation of Coronary Microvessels**

It has been reported that the relaxation response of rat thoracic aorta to acetylcholine (presumably NO-mediated) was significantly attenuated after a 3-day peritoeinal infusion of a subpressor dose of Ang II.9 Furthermore, intracoronary infusion of Ang II (3 nmol/L), which did not affect resting coronary blood flow, significantly attenuated the increase in blood flow to subsequent intracoronally injected adenosine in the dog.24 In the latter study, it is possible that Ang II may have affected the coronary microcirculation, because preferential action of adenosine on the small coronary arterioles has been previously reported.25 Although these two studies implicate that Ang II may modulate vascular function independently of its direct vasomotor action, the receptor subtypes and the underlying mechanisms responsible for the inhibitory effect of Ang II remain elusive. In view of the fact that upregulation of the RAS plays a detrimental role in the progression of many cardiovascular diseases (eg, heart failure, diabetes, atherosclerosis, hypertension, and myocardial infarction)26 and that adenosine is a putative metabolic vasodilator contributing to the regulation of coronary blood flow during ischemia and metabolic stress,27 the interaction of Ang II and adenosine in the coronary microcirculation may become a major factor influencing coronary perfusion during disease states or under cardiovascular stress.

We have previously demonstrated that the endothelial release of NO and the activation of smooth muscle KATP channels underlie the mechanism of coronary arteriolar dilation to adenosine.12 In agreement with previous findings, the endothelial NO component was evident in the present study because L-NMMA and endothelial denudation attenuated the adenosine-induced vasodilation. Interestingly, in a similar manner, a 60-minute incubation of endothelium-intact coronary arterioles with Ang II, which did not affect resting diameter, inhibited the adenosine-induced vasodilatory response. In contrast, Ang II did not affect the dilation of either endothelium-denuded or L-NMMA–pretreated vessels, indicating that Ang II inhibited the endothelium-dependent NO-mediated dilation to adenosine. This inhibitory effect seemed to be specific to Ang II because a subthreshold concentration...
(10 nmol/L) of acetylcholine, a potent vasoconstrictor in porcine coronary resistance vessels,29 did not elicit impairment of adenosine-induced vasodilation (n=3, data not shown). Activation of AT1 receptors is likely involved in this inhibitory process because losartan, but not PD 123319, prevented the effect of Ang II. These contentions are additionally supported by the results that Ang II nearly abolished the adenosine-stimulated NO production and losartan preserved the release of NO. It seems that the inhibitory effect of Ang II was selective for the endothelial NO pathway because Ang II also inhibited vasodilation to other endothelium-dependent NO-mediated agonists (ie, serotonin13,30 and ionomycin30) (data not shown) without affecting endothelium-dependent vasodilations to cytochrome P-450 monoxygenase activator bradykinin33 and cyclooxygenase activator arachidonic acid,21 as shown in the present study. Furthermore, the inhibition of smooth muscle KATP channels is also unlikely, because Ang II affected neither adenosine-induced dilation of endothelium-denuded vessels nor vasodilation to KATP channel activator pinacidil. Thus, the present results support previous findings in vivo9,24 and additionally demonstrate that Ang II can directly impair adenosine-induced NO-mediated dilation in the coronary microcirculation via activation of arteriolar AT1 receptors.

**Ang II–Induced Superoxide Production in Coronary Microvessels**

Because Ang II can stimulate cellular production of superoxide and the latter is an NO scavenger, a putative mechanism contributing to the impairment of NO-mediated vasodilation via AT1 receptor–mediated superoxide production should be considered. Indeed, we found that the impaired adenosine-induced dilation by Ang II was reversed by a membrane-permeable superoxide scavenger, TEMPOL.15 The effect of TEMPOL seems to be specific, because this superoxide scavenger did not affect basal tone or vasodilations to sodium nitroprusside and pinacidil (data not shown). Similar to TEMPOL, another membrane-permeable superoxide scavenger, Tiron,30 also restored the normal vasodilatory response to adenosine (data not shown). Interestingly, pyrogallol, a superoxide generator,17 inhibited the vasodilation to adenosine in a similar manner as denudation and L-NMMA and also mimicked the inhibitory effect of Ang II. These results not only demonstrate that superoxide generation is capable of impairing NO-mediated coronary arteriolar function but also implicate that this oxidative free radical might be involved in the Ang II–induced vascular dysfunction. Furthermore, it seems that the Ang II–induced superoxide was generated predominantly by NADPH oxidase, because the respective inhibitor apocynin16 restored coronary arteriolar dilation to adenosine.

Although previous studies have shown that Ang II increases the generation of superoxide in cultured endothelial7,8 and vascular smooth muscle cells,31 there is a disagreement in the receptor subtypes (AT1 or AT2) involved in the superoxide production.7,8 Furthermore, it is unclear whether Ang II is able to induce superoxide production in the intact microvessels. In the present study, the increased DHE staining for superoxide, which is losartan sensitive and TEMPOL reversible, in both endothelial and smooth muscle layers of Ang II–treated vessels indicates that Ang II, at a nonvasoactive concentration, is capable of generating superoxide in the vascular wall through AT1 receptor activation. It is worth noting that the concentration of Ang II used in those cultured cell studies (1 to 200 nmol/L)7,8,31 was much higher than that used in the present study (0.1 nmol/L). It has been reported that the estimated physiological and pathophysiological plasma levels of Ang II in humans are ≈10 pmol/L and 0.1 nmol/L, respectively.7,23 Although our results suggest that this pathophysiological level of Ang II is sufficient to promote AT1 receptor–mediated NO deficiency, it is not yet known whether vasoactive levels of Ang II that were observed in the present study (ie, 1 nmol/L to 1 μmol/L) are attained in the coronary microcirculation in vivo. Nevertheless, a functional RAS has been shown to exist in the heart,54 and it is speculated that activation of this system during physiological stress could potentially provide a greater transient increase in local Ang II for flow regulation.

In conclusion, we have demonstrated that Ang II is a vasoactive agent that causes coronary arteriolar constriction (via AT1 receptors) or dilation (via AT2 receptors). A low level of Ang II, without eliciting any vasomotor activity, selectively inhibits NO production and endothelium-dependent NO-mediated dilation in response to adenosine. The activation of AT1 receptors and subsequent generation of superoxide by NADPH oxidase are responsible for the adverse effect of Ang II. Because adenosine and Ang II can be concomitantly released during myocardial ischemia or stress, it is possible that the released Ang II may counteract the vasodilatory function of adenosine by impairing NO-mediated vasomotor activity. It is speculated that a moderate elevation of Ang II as a result of chronic activation of the RAS in hypertension and heart failure may potentially mitigate the role of NO in coronary flow regulation and thus exacerbate the already deteriorated cardiac function.

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