In vivo visualization of subendocardial arteriolar response in renovascular hypertensive hearts

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In vivo visualization of subendocardial arteriolar response in renovascular hypertensive hearts. Am J Physiol Heart Circ Physiol 284: H1785–H1792, 2003. First published January 9, 2003; 10.1152/ajpheart.00819.2002—Time-sequential responses to endothelium-dependent and -independent vasodilators and angiotensin-converting enzyme (ACE) inhibitors were studied in the subendocardial arterioles (Endo) of canine renovascular hypertension (HT) compared with subepicardial arterioles (Epi; both <120 μm) by charge-coupled device intravital microscope. Vascular responses to acetylcholine, papaverine, and cilazaprilat were compared between normotensive (NT) and HT dogs [4 wk and 12 wk of HT (4wHT and 12wHT)] (model VS600, IDC) with room air, supplemented with 100% oxygen. Aortic pressure (AoP) and LV pressure were measured (NIH Publication No. 85-23, Revised 1996). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
sured with an 8-Fr pigtail double manometer catheter (model SPC-784A, Millar). The proximal portion of the left anterior descending coronary artery (LAD) was isolated and a transonic flow probe (model T206, Transonic Systems; Ithaca, NY) was placed around the vessel. The heart rate was kept constant at 100 beats/min during the experiment by right ventricular pacing after atrioventricular node blocking with 37% formaldehyde.

**Needle-probe intravital microscope**. Details of the needle-probe intravital microscope have been previously described (29). Briefly, the needle probe (4.5 mm diameter) contains a gradient index lens surrounded by light guide fibers and a double-lumen sheath. A doughnut-shaped balloon on the tip avoids direct compression of the vessels by the needle tip. To obtain a clear image of the vessels, blood between the tip of the needle probe and the endocardium inside the doughnut was flushed away with a warm buffer solution injected through a microtube of the sheath.

**Measurements of arteriolar diameters**. The needle probe was introduced into the endocardium of LV through an incision in the left atrial appendage via the mitral valve. When a clear arteriolar image was obtained, the operator kept the probe position on the vessel manually. The vascular images were recorded by using a syringe pump. The vascular responses of Endo arterioles were evaluated. Third, after reverting to Endo. There was a significant difference was observed only between NT and 12wHT (P < 0.05), but a decreasing tendency probably due to wider scattering. Figure 2 is a comparison between Endo and Epi. The vasodilation of Endo in 4wHT and 12wHT was smaller than that of Epi (Fig. 2, B and C; P < 0.01), but no significant difference in the NT group (Fig. 2A). As for the vascular-size-dependent vasodilations, smaller vessels dilated more in both Endo and Epi. Collectively, endothelium-dependent vascular responses of arterioles were impaired in 4wHT and 12wHT were evaluated (see Fig. 5C). Fifth, when coronary blood flow and blood pressure recovered the baseline, the ACE inhibitor cilazaprilat (10.0 μg/kg per min ic) was continuously infused and Endo arteriolar responses were evaluated (see Fig. 5D). Finally, after reverting of the hemodynamics, the ACE inhibitor cilazaprilat was continuously infused again and Epi arteriolar responses were evaluated. All drugs were obtained from Sigma, except for cilazaprilat, which was purchased from Eisai (Tokyo, Japan).

**Statistical analysis**. Data were reported as means ± SE. The difference in effects of acetylcholine, papaverine, and an ACE inhibitor on subendocardial arteriole versus subepicardial arteriole was tested by a multiple regression analysis with the use of a model, in which the change in diameter was set as a dependent variable (y) and vascular size as an explanatory variable (x), whereas the statuses of 4wHT and 12wHT were set as the dummy variables D1 and D2, respectively: y = a0 + a1x + a2D1 + a3D2, where a0–a3 are partial regression coefficients. Student’s t-test was used for both paired and unpaired comparisons. The criterion for statistical significance was P < 0.05.

**RESULTS**

**Hemodynamic parameters**. The LV mass per body mass was 4.9 ± 0.3 g/kg in the NT group (n = 19), 6.6 ± 0.2 g/kg in 4wHT (n = 15, P < 0.01 vs. NT), and 6.8 ± 0.5 g/kg in 12wHT (n = 6, P < 0.05 vs. NT). LV wall thickness was 9 ± 1 mm in the NT group, 12 ± 1 mm in 4wHT (P < 0.01 vs. NT), and 14 ± 1 mm in 12wHT (P < 0.01 vs. NT). LV mass and wall thickness were not different between 4wHT and 12wHT. The systolic AoP (SBP; 4wHT, 145 ± 6 mmHg; 12wHT, 179 ± 12 mmHg) and diastolic AoP (DBP; 4wHT, 109 ± 5 mmHg; 12wHT, 137 ± 16 mmHg) of HT were higher than those of the NT group (SBP, 103 ± 4 mmHg, and DBP, 76 ± 4 mmHg, P < 0.05 and P < 0.01 vs. both hypertensive groups, respectively). SBP and DBP were different between 4wHT and 12wHT (both P < 0.05).

Table 1 lists the baseline hemodynamics after the dogs were stabilized with anesthesia and prepared for surgery. SBP and DBP were not significantly different between NT and both HT groups. LV end-diastolic pressure in HT slightly increased compared with NT. The LAD flow response during each intervention increased significantly from baseline values.

**Endothelium-dependent vasodilator response**. Figure 1A compares endothelium-dependent vasodilation in Endo. There was a significant difference between NT and 4wHT, and between NT and 12wHT (both P < 0.01), but not between 4wHT and 12wHT. In Epi (Fig. 1B), a significant difference was observed only between NT and 12wHT (P < 0.05), but a decreasing tendency without significance between NT and 4wHT (P = 0.07), probably due to wider scattering. Figure 2 is a comparison between Endo and Epi. The vasodilation of Endo in 4wHT and 12wHT was smaller than that of Epi (Fig. 2, B and C; P < 0.01), but no significant difference in the NT group (Fig. 2A). As for the vascular-size-dependent vasodilations, smaller vessels dilated more in both Endo and Epi. Collectively, endothelium-dependent vascular responses of arterioles were impaired in

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Table 1. Baseline hemodynamics during evaluation of Endo and Epi

<table>
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<tr>
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<th>Acetylcholine</th>
<th>Papaverine</th>
<th>Cilazapril</th>
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<tr>
<td>before</td>
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<td>Before</td>
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<tr>
<td>systolic blood pressure, mmHg</td>
<td>n</td>
<td>Before</td>
<td>After</td>
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<tr>
<td>Normal</td>
<td>16/10</td>
<td>97 ± 6</td>
<td>93 ± 7</td>
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<tr>
<td>4wHT</td>
<td>15/9</td>
<td>115 ± 7</td>
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<tr>
<td>12wHT</td>
<td>15/5</td>
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<td>109 ± 4</td>
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<td>mean blood pressure, mmHg</td>
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<td>Before</td>
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<tr>
<td>Normal</td>
<td>16/10</td>
<td>69 ± 6</td>
<td>68 ± 6</td>
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<tr>
<td>4wHT</td>
<td>15/9</td>
<td>83 ± 7</td>
<td>80 ± 6</td>
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<td>12wHT</td>
<td>15/5</td>
<td>88 ± 5</td>
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<td>LV end-diastolic blood pressure, mmHg</td>
<td>n</td>
<td>Before</td>
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<tr>
<td>Normal</td>
<td>16/10</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
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<td>4wHT</td>
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<td>n</td>
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<tr>
<td>Normal</td>
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<td>76 ± 5</td>
<td>81 ± 4</td>
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<tr>
<td>12wHT</td>
<td>15/5</td>
<td>73 ± 6</td>
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Values are means ± SE; n, no. of vessels vs. number of dogs. Endo, subendocardial arterioles; Epi, subepicardial arterioles; before, before administration of vasodilators; after, after administration of vasodilators; LV, left ventricular; 4wHT and 12wHT, 4th and 12th wk of hypertension, respectively. *P < 0.05 vs. normotension; †P < 0.01 vs. normotension; ‡P < 0.05 vs. before acetylcholine, papaverine, and cilazapril; §P < 0.01 vs. before acetylcholine, papaverine, and cilazapril.
both 4wHT and 12wHT in Endo, but only significant in 12wHT in Epi.

**Endothelium-independent vasodilator response.** Figure 3, A and B, compares endothelium-independent vasodilation in the NT group with both HT groups in Endo and Epi. There was no significant difference between NT and 4wHT, but between NT and 12wHT (both \( P < 0.01 \)), nor between 4wHT and 12wHT (normal, \( n = 16/10 \); 4wHT, \( n = 15/5 \); 12wHT, \( n = 15/6 \)). However, in Epi (NT, \( n = 26/12 \); 4wHT, \( n = 29/14 \); 12wHT, \( n = 15/6 \)), a significant difference was observed only between NT and 12wHT (\( P < 0.05 \)).

**DISCUSSION**

The major findings from the present study were the following: 1) at 4wHT, the endothelium-dependent arteriolar response of Endo was impaired, but the endothelium-independent response was preserved; 2) at 12wHT, both the endothelium-dependent and independent responses of Endo were impaired, but only the former was impaired in Epi; and 3) dilation after administration of the ACE inhibitor was not altered transmurally in the course of HT. Our results and discussion depend critically on several factors: 1) critique of experimental model and methodology; 2) impairment of coronary vasodilatory reserve of Endo with LV hypertrophy; 3) time course of impairment of endothelium-dependent and independent vasodilator response; 4) the effect of the ACE inhibitor; and 5) clinical implications.

**Critique of experimental model and methodology.** We used Goldblatt HT dog model by application of 2K2C...
In our renovascular model, development of 2K2C HT was associated with a marked increase in plasma renin activity, plasma angiotensin I, and plasma angiotensin II, followed by moderate increases during the maintenance of HT (22). We confirmed significant increases in SBP and DBP of both 4wHT and 12wHT. However, as for baseline hemodynamics during experiments, aortic pressure decreased in the hypertensive animals probably due to the anesthesia and surgical preparation. We focused our observation on the arterioles in both Endo and Epi with diameters of 45–120 μm because of the limitation of spatial resolution of our needle-probe videomicroscope. The vasodilation by acetylcholine and papaverine was attenuated in the course of our model hypertension, especially in Endo without any reduction in mean coronary flow. The discrepancy between the vessels and flow responses may be due to the greater involvement of metabolic autoregulation of the smaller arterioles below our spatial resolution and also due to transmural difference of vasodilation. Tomanek et al. (24) reported that the maximum flow by adenosine, which dilates preferentially smaller arterioles, was not different between control and 7-mo-old one-kidney, one-clip HT canine models, supporting our interpretation. In our experimental setup, the vascular diameter pulsation in Endo

Fig. 3. Endothelium-independent vasodilator response. Percentage of arteriolar diameter changes of Endo (A) and Epi (B) to papaverine in NT (Endo, n = 12/6; Epi, n = 17/10), 4wHT (Endo, n = 12/8; Epi, n = 19/12) and 12wHT (Endo, n = 15/5; Epi, n = 12/6). There is no significant change in 4wHT in both Endo and Epi from control. The arteriolar response in 12wHT was impaired only in Endo (both P < 0.01, vs. NT and 4wHT).

Fig. 4. Vascular response to angiotensin-converting enzyme (ACE) inhibitor. Percentage of arteriolar diameter changes of Endo (A) and Epi (B) to cilazaprilat in NT (Endo, n = 13/7; Epi, n = 12/7), 4wHT (Endo, n = 13/8; Epi, n = 19/12) and 12wHT (Endo, n = 13/5; Epi, n = 12/6). There is no significant difference among three groups in both Endo and Epi, and also no transmural difference.
during a cardiac cycle was 21% in normal, 17% in 4wHT \( (P < 0.05 \text{ vs. control}) \), and 15% in 12wHT \( (P < 0.05 \text{ vs. control}) \). The decreased arteriolar diameter pulsation in Endo with HT may be mainly because the systolic blood pressure is reduced remarkably by the anesthesia and surgical preparation. The reduced compression may allow systolic myocardial inflow to some extent, contributing to discrepancy between the mean flow and diastolic vascular response. Because the systolic vascular compression in Endo must be much higher in conscious HT animals, their systolic myocardial inflow and mean coronary flow may be reduced with the decrease in diastolic vascular responses. On the other hand, abnormal flow responses of human coronary circulation to acetylcholine in patients with HT were well documented, probably due to the longer time course of HT in humans (26).

Acetylcholine and papaverine are well-established endothelium-dependent and -independent vasodilators. Microvascular responses were measured after an intracoronary administration \( (1.0 \mu g/kg) \) and bolus injection \( (1 \text{ mg}) \), respectively, because we found that these procedures and doses produced transient maximal microvascular response to each agent without any systemic hemodynamics effects, as reported by Defily et al. (6). The intracoronary administration of cilazaprilat \( (10.0 \mu g/kg) \) increased coronary flow without any changes in the systemic hemodynamic as Kitakaze et al. (15) indicated.

Kanatsuka et al. (14) reported that microvascular resistance in vessels \( (<150 \mu m) \) was twofold higher in HT cats compared with NT cats, whereas arterial resistance in vessels \( (>150 \mu m) \) was similar in HT and NT. Thus the present study focused on arterioles \( <120 \mu m \). Another reason for the selection of arterioles \( (<120 \mu m) \) was practical difficulty in finding larger arterioles \( (>120 \mu m) \) in Endo.

The methodological validity in the present study has been confirmed previously (8, 29). However, holding the needle probe on a fixed visual field of the endocardial surface for several minutes is usually technically difficult. Accordingly, we measured arteriolar diameters for \( \pm 2 \text{ min after vasodilator infusion to detect the maximal vasodilation, which had been validated by observation over longer periods. The spatial resolution} \)}
of this system is ~5 μm for ×200 magnification (29). The maximum depth of field is ~250 μm.

**Impairment of coronary vasodilatory reserve of Endo with LV hypertrophy.** Reduction in coronary flow reserve, especially in Endo is a possible mechanism for the impaired cardiac function in LV hypertrophy. Indeed, Vatner et al. (28) showed the importance of impaired coronary reserve in Endo as a mechanism for the diastolic dysfunction in a hypertrophied heart exposed to mechanical stresses. Harrison et al. (7) and Jeremy et al. (13) also indicated that HT and LV hypertrophy were associated with a profound impairment of the lower range of autoregulation in Endo. Hittinger et al. (10) and Bishop et al. (3) suggested that hemodynamic factors, i.e., compressive forces, were more important for reduced endocardial coronary reserve than structural alterations in LV hypertrophy caused by aortic stenosis. Thus the elevated LV end-diastolic pressure and wall stresses with increased systolic stresses, particularly in Endo, may cause abnormal endocardial coronary reserve.

By using our needle lens probe charge-coupled device videomicroscope, we found that Endo arterioles were more compressed at end systole compared with Epi under control conditions in pigs and dogs (8, 29). Compressive forces affect intramyocardial coronary circulation (1, 11). The increased systolic compression may be augmented in hypertensive hearts. Collectively, it is likely that coronary vasodilatory reserve of hypertensive hypertrophic hearts was more impaired in Endo than Epi.

**Time course of impairment of endothelium-dependent and -independent vasodilator response.** It has been demonstrated that acetylcholine-induced relaxation is impaired in resistance arteries of experimental renovascular HT (17, 19) as well as other hypertensive models. Endothelium-dependent arteriolar response of Endo was already attenuated at 4wHT, whereas those of Epi were impaired at 12wHT (Fig. 2). The degree of the impairment is also greater in Endo than in Epi. It is known that nitric oxide (NO) release is inhibited in a pressure-dependent manner (9). In HT, systolic intramyocardial pressure should be increased with increasing LV pressure, and this trend is greater in Endo than in Epi, leading to higher intravascular pressure. Thus pressure-induced loss of NO release may be greater in Endo, reducing vascular dilation in Endo to which endothelium-derived NO significantly contributed (30).

The response to papaverine was impaired only in Endo of 12wHT. In moderate canine LV hypertrophy induced by renovascular HT for 6 wk and 7 mo, Tomank et al. (23, 24) could not find any increase in the medial area of intramyocardial arteries nor any significant architectural difference. Thus in the renovascular hypertensive model, impaired endothelium-independent vascular responses in 12wHT may be the consequence of functional alterations in the microvascular smooth muscle. In addition, increased production and/or release of endothelium-dependent contractile factors (16, 18), such as angiotensin II, prostaglandin H₂, thromboxane A₂, and/or endothelin-1 with augmented sensitivity of vascular smooth muscles to vasoconstrictor stimuli in HT may lead to impaired vascular response.

**Effect of ACE inhibitor.** Several studies (21, 31) have provided evidence for acute antihypertensive action of ACE inhibitors in different models of HT, which operates by dilating both canine coronary conductance and resistance arteries. In the present study, the vasodilation to the ACE inhibitor was unaltered, even in Endo in the course of HT (Figs. 4 and 5). What is the mechanism for unaltered vasodilation by the ACE inhibitor in the course of HT? To produce high blood pressure in dogs, we used our earlier 2K2C model. In this model, the elevation of blood pressure was associated with increases in plasma renin and angiotensin I and II, thus indicating activation of the renin-angiotensin system. Although the degree of angiotensin II increment became moderate during the maintenance of HT after initial remarkable augmentation, the vasodilatory response to the ACE inhibitor might be greater in the renovascular HT model. Thus an increased constrictor influence of angiotensin may override the papaverine-induced vasodilation. This may be a major reason for unaltered responses to the ACE inhibitors transmurally in the course of HT.

Kinin-NO-induced coronary vasodilation may be also related to the ACE inhibitor-induced vasodilation. Blockade of B₂ kinin receptors has been shown to attenuate the hypotensive effect after a bolus injection of ACE inhibitor in two-kidney, one-clip hypertensive Wistar rats (2). Kitakaze et al. (15) demonstrated that an intracoronary infusion of cilazaprilat increased coronary flow in ischemic condition, especially in Endo with increase in bradykinin concentration and cGMP, suggesting that the kinin-NO effects are more prominent in Endo. The endothelial NO effect due to an ACE inhibitor might be partly modulated by vascular superoxide production. Angiotensin II has been shown to stimulate the NAD(P)H oxidase in smooth muscle cells, resulting in increased generation of superoxide that degrades NO (5). Actually, cilazaprilat prevented myocardial reactive oxygen species in Dahl salt-sensitive rats (27). However, because oxidative stress was not involved in the stage of compensatory hypertrophy (27), as in the present study, but was in the stage of heart failure, its contribution in our animal models is likely to be minor, if present at all.

**Clinical implications.** This study discusses the results in which endothelium-dependent vasodilation was markedly impaired in the coronary microvessels of patients with HT and LV hypertrophy (25). The present study also indicated early (4th wk) impairment of endothelium-dependent vasodilation, but later (12th wk) involvement of endothelium-independent vasodilation, especially in Endo in the canine renovascular HT model. Thus the early control of HT may relieve the vascular dysfunction, especially endothelium-dependent impairment of vasodilation in Endo. ACE inhibitors may be one of the preferable treatments against renovascular HT to preserve both endothelium-depen-
dent and -independent vasodilation. The homogenous myocardial blood flow distribution caused by ACE inhibitors (20) may be also beneficial to myocardial perfusion.

In conclusion, at 4wHT, the endothelium-dependent response of Endo was impaired. At 12wHT, both the endothelium-dependent and -independent responses of Endo were disturbed in the renovascular hypertensive hypertrophic canine model. These sequential changes may be related to the degree of decrease in coronary flow reserve of Endo in the course of LV hypertrophy. Larger impairment of coronary vascular response in Endo indicates the crucial role of higher hypertensive mechanical stress there. The vasodilatory responses to ACE inhibitor were preserved at 4wHT and 12wHT in both Endo and Epi. The mechanisms may be related to the effects of ACE inhibitors on angiotensin II and kinin-NO cascades.

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