Diastolic properties in canine hypertensive left ventricular hypertrophy: Effects of angiotensin converting enzyme inhibition and angiotensin II type-1 receptor blockade

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Abstract

Objective: Angiotensin II has been suggested to be involved in the pathogenesis of diastolic dysfunction in left ventricular hypertrophy (LVH). The purpose of this study was to assess the effects of enalaprilat and L-158,809, an angiotensin II type-1 receptor antagonist, on LV diastolic function in 16 normal control dogs and 20 LVH dogs with perinephritic hypertension. Methods: LV hemodynamics was studied before and after intravenous injection of enalaprilat (0.25 mg/kg) or L-158,809 (0.3 mg/kg). The hemodynamic data were analyzed in relation to the changes in myocardial blood flow (measured by radioactive microspheres) and in the circulating angiotensin II and norepinephrine levels. Results and Conclusions: At baseline, significant increases were observed for LV/body weight ratio as well as LV systolic and end-diastolic pressures in the LVH dogs (all P < 0.01 vs. the control group). In addition, LV relaxation time constant was prolonged and the chamber and myocardial stiffness constants were increased (P < 0.01) in the LVH dogs, suggesting an impairment of LV diastolic function. Administration of enalaprilat or L-158,809 improved LV stiffness constants in the LVH dogs (P < 0.05). The diastolic LV pressure–diameter relation shifted downwards in the LVH dogs whereas diastolic distensibility was not altered in the control dogs. Although the circulating angiotensin II levels were significantly decreased by enalaprilat in the LVH dogs, they did not correlate with the changes in the stiffness constants. Furthermore, the alterations of LV diastolic properties in the LVH group could not be attributed to myocardial perfusion, which was rather decreased by administration of enalaprilat and L-158,809. These results suggest that angiotensin II, particularly at the local level, is involved in the pathogenesis of diastolic dysfunction in pressure-overload LVH. The data also support the concept that ACE inhibitors and angiotensin II receptor blockers are potentially beneficial in the treatment of the hypertrophied heart.

Keywords: Renin–angiotensin–aldosterone system; Angiotensin II; Diastolic function; ACE inhibitors; Dog, anesthetized; Hypertrophy

1. Introduction

There is increasing evidence that angiotensin II plays an important role in the pathogenesis of diastolic dysfunction in pressure-overload left ventricular hypertrophy (LVH) [1–4]. Alterations of myocardial relaxation and diastolic properties exist in the hypertrophied left ventricle [5,6], which has recently been suggested to be related to the enhanced activity of the renin–angiotensin system [1–4]. Angiotensin II promotes influx and release of calcium, and increases its intracellular level in myocytes [7–9]. This effect of angiotensin II can be detrimental to cardiac homeostasis in the hypertrophied myocardium and may aggravate its intrinsic abnormalities of sarcoplasmic calcium re-uptake and myocardial inactivation [10,11].

The impaired myocardial inactivation causes residual cross-bridge attachment persisting during diastole (active elasticity), which may result in alterations of diastolic
myocardial properties and chamber distensibility of the left ventricle [12,13]. Although the changes in LV diastolic properties have been considered to be mainly due to chronic structural remodeling of the ventricular wall [2,14], angiotensin II also may directly affect the active elasticity of diastolic properties in pressure-overload LVH.

To test this hypothesis, we studied separately the effects of acute ACE inhibition and angiotensin II receptor blockade on LV diastolic properties in a canine model of pressure-overload LVH due to perinephric hypertension [15–17].

2. Methods

2.1. Study groups

The study groups consisted of 36 adult mongrel dogs (16 normal control dogs and 20 LVH dogs with perinephric hypertension). There was no difference in body weight between the control and LVH groups (21 ± 1 vs. 21 ± 2 kg). Eight control dogs and 10 LVH dogs received enalaprilat (Renitec® i.v., Merck Sharp and Dohme, Brussels, Belgium) that was infused intravenously (i.v.) as a bolus at a dose of 0.25 mg/kg body weight (groups, CTL-E and LVH-E). The other 8 control dogs and 10 LVH dogs received a nonpeptide, Type 1 receptor-selective angiotensin II antagonist, L-158,809 (5,7-dimethyl-2-ethyl-3-[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-3H-imidazo[4,5-β]pyridine, Merck Research Laboratories, Blue Bell, PA), at a dose of 0.30 mg/kg bolus i.v. (groups, CTL-L and LVH-L). It is known that maximal blockade of angiotensin I pressor response can be achieved by 0.25 mg/kg of enalaprilat [18]. Our pilot experiments showed that a similar reduction in the systemic blood pressure was achieved by 0.25 mg/kg of enalaprilat or by 0.3 mg/kg of L-158,809 and that higher dose of either drug did not produce additional vasodilatation.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985) and also with the guidelines of the Committee for Animal Research of the Belgian Fonds National de la Recherche Scientifique Médicale.

2.2. Preparation of hypertensive LVH dogs

Twenty dogs underwent surgery for creating perinephric hypertension by wrapping the left kidney under general anesthesia with intravenous thiopental sodium (5 mg/kg) and enflurane gas (2–3 vol%), as described previously [15]. The dogs were allowed for the recovery and development of hypertension during the following 6 weeks. At the time of the experiment, all of the dogs had recovered from the surgery. Symptoms of heart failure were absent, and plasma creatinine and urea nitrogen levels were within normal ranges in all dogs.

2.3. Experimental procedures

On the day of the experiment, the dogs were intubated and ventilation was maintained under general anesthesia with intravenous pentobarbital sodium (20 mg/kg). The chest was opened at the left fifth intercostal space, the pericardial sac was incised, and the heart was exposed in the pericardial cradle. A high-fidelity micro-manometer (JSI 0400, Serel, Aeuillez, Belgium) was introduced into the LV cavity through the apex. A Tygon catheter, filled with heparinized saline, was introduced into the left atrium for the introduction of radioactive microspheres. A polyethylene band (5 mm in width) was placed around inferior vena cava just above the diaphragm for vena caval occlusion. One pair of 5-MHz ultrasonic crystals (Triton Technology, San Diego, CA) was implanted at opposing sites on the anterior and posterior LV epicardial surfaces at midventricular level to measure antero-posterior external (epicardial) diameter. Another pair of ultrasonic crystals was implanted to measure wall thickness in the anterior wall of the midventricle, adjacent to the anterior crystal for the diameter. An 8F USCI catheter, filled with heparinized saline, was advanced to the descending thoracic aorta via the right femoral artery and was connected to a Gould P23 ID transducer with zero reference at the level of the left ventricle. Another Tygon catheter was inserted into the right femoral vein for volume infusion.

After the baseline hemodynamic data were obtained, 100 ml of saline warmed at 37°C was slowly injected into the femoral vein. This saline volume was determined in the pilot experiments to be enough to increase LV end-diastolic pressure and diameter to the steep portion of the diastolic compliance curve, which gave us appropriate data points to calculate the stiffness constants. When the increases in LV diastolic pressure and dimensions reached the plateau levels, inferior vena cavocaval occlusion was performed until stable minimal dimensions were observed (Fig. 1), which was accomplished within 10–20 s in all dogs. Then the occlusion was released. During vena cavocaval occlusion, respiration was suspended at end-expiration to avoid its effect on diastolic pressure and dimensions. When the hemodynamic conditions were judged to have returned to the pre-occlusion basal levels, enalaprilat or L-158,809 was injected intravenously over 5 min. After the administration of the drug, the second set of the steady-state hemodynamic data was obtained, and volume infusion and subsequent vena cavocaval occlusion were repeated in the same manner. The whole experimental procedure was accomplished within 1 h for all animals.

2.4. Hemodynamic data processing and LV function analysis

Analog hemodynamic signals were digitized at 2 ms intervals with 100 Hz filtering, and were processed by a Hewlett-Packard A900 computer [19]. A first derivative of
LV pressure (dP/dt) was obtained by digital differentiation of the pressure data. The end-diastole was defined at the peak of R-wave on the electrocardiogram, and the end-systole was defined at the time of peak(−dP/dt). LV antero-posterior internal diameter was derived by the external diameter minus (2 × wall thickness). Fractional shortening was obtained by end-diastolic internal diameter minus end-systolic internal diameter divided by end-diastolic internal diameter. LV relaxation rate was assessed by time constant during the first 80 ms after peak(−dP/dt). The time constant τ was derived from the regression of dP/dt versus LV pressure: dP/dt = (−1/τ) · (P − PB), where P is LV pressure and PB is variable pressure asymptote [20]. The correlation coefficients for τ calculation were 0.993 ± 0.001 in the control group and 0.984 ± 0.004 in the LVH group.

To assess LV chamber diastolic properties at the midventricular level, the data for LV end-diastolic pressure (EDP) and the corresponding internal diameter during vena caval occlusion were fitted by a monoexponential relation: 
P = α_ε e^{β_p t} + γ_c,

where P is pressure, D is internal diameter, and α_ε, β_p, and γ_c are constants. The three constants were determined by a nonlinear curve fitting program, and β_p is the chamber stiffness constant. The correlation coefficient for the monoexponential fitting was 0.987 ± 0.005 for the control group and 0.980 ± 0.007 for the LVH group.

The midwall strain (ε) was calculated by using a natural strain definition, ε = ln(l/l_0), where l is the midwall circumference, calculated as π(D + h), and l_0 is the unstressed circumference at LV pressure = 0. The l_0 was determined from the monoexponential relation between LVEDP and midwall circumference during vena caval occlusion. Then, end-diastolic stress and strain data were fitted by the equation: 
σ = σ_m (e^{β_m t} − 1).

The constants α_m and β_m were determined, and β_m is the myocardial stiffness constant [21]. The correlation coefficient for this relation was 0.988 ± 0.004 for the control group and 0.987 ± 0.005 for the LVH group.

2.5. Myocardial blood flow

Myocardial blood flow to the left ventricle was determined at baseline and after the drug injection by using radioactive microspheres (16 μm diameter, Du Pont NEN Products, Dreieich, Germany) labeled with ^{57}Co, ^{113}Sn, ^{85}Sr, and ^{99}Nb. Microspheres were injected into the left atrium after the steady-state hemodynamic data collection. Reference arterial samples were also obtained at a constant rate of 10 ml/min. After the experiment, potassium chloride was injected into the animals under anesthesia (pentobarbital 20 mg/kg). The left ventricle was excised and weighed. At least two transmural sections (about 2 g) of the ventricular wall were obtained and each section was divided into endocardial and epicardial halves. Regional blood flow was determined from the radioactivity of tissue and reference blood samples by standard methods [19].

2.6. Plasma angiotensin II and norepinephrine levels

Blood samples for the measurement of plasma angiotensin II and norepinephrine were collected at baseline and after the drug injection when its hemodynamic effect on blood pressure reached the maximal level (10–20 min after the injection). Angiotensin II was measured after plasma extraction by radioimmunoassay, using specific antibodies and synthetic peptides (Peninsula, Belmont, CA). Intra- and interassay coefficients of variation were 6.5 and 8.5%, and the sensitivity of the radioimmunoassay, defined as 10% tracer displacement, was 1.9 pg per tube. Norepinephrine was measured by high-performance liquid chromatography with electrochemical detection and a cation exchange analytical column (Bio-Rad Clinical Division, Hercules, CA). Intra- and interassay coefficients of variation were 6.6 and 12%, respectively.

2.7. Statistical analysis

Data are presented as mean ± s.e.m. Analysis of variance was employed to assess changes in hemodynamic
3. Results

3.1. Effects of hypertension on the heart weight

The LV/body weight ratio and LV relative wall thickness (a ratio of end-diastolic wall thickness to LV internal diameter) were significantly greater in the LVH group than in the control group (Table 1). The right ventricular/body weight ratio was not different between the two groups.

3.2. Baseline LV hemodynamic data (Table 1)

Table 1 also summarizes the baseline LV hemodynamics in the pooled control (n = 16) and LVH (n = 20) groups. When compared with the control group, LV systolic and end-diastolic pressures as well as peak+/-dP/dt were significantly greater in the LVH group. Although LV internal diameters were smaller in the LVH group, no significant difference was observed for the fractional shortening between the two groups. In the LVH group, relaxation time constant \( \tau \) was prolonged and \( P_a \) was smaller, and LV stiffness constants \( \beta_c \) and \( \beta_m \) were also increased. These findings suggest an impairment of LV relaxation and diastolic properties.

3.3. Effects of enalaprilat and L-158,809 on LV hemodynamics (Table 2)

Heart rate was significantly decreased in the LVH-E group but not in the CTL-E group after enalaprilat injection. In contrast, heart rate was increased by L-158,809 injection in the CTL-L group but did not significantly change in the LVH-L group. However, enalaprilat and L-158,809 caused similar decreases in LV systolic pressure and mean aortic pressure in the control groups as well as in the LVH groups. No significant change was observed for LV diameter or fractional shortening.

3.4. Effects of enalaprilat and L-158,809 on LV diastolic properties (Tables 2 and 3)

LVEDP after volume loading with a 100 ml of saline (LVEDP- VL) was not altered by enalaprilat or L-158,809 in the control groups (Table 2), and no change was observed in the diastolic LV pressure–diameter relation (Fig. 2). In the LVH groups, however, LVEDP- VL was signifi-
cantly lowered by enalaprilat and by L-158,809 (both $P < 0.01$) without substantial difference in the corresponding end-diastolic internal diameter. Diastolic LV compliance curve was shifted downward by enalaprilat (Fig. 2) and also by L-158,809 (Fig. 3) in the LVH group. LV stiffness constants $\beta_c$ and $\beta_m$ did not change significantly after injection of enalaprilat or L-158,809 in the control group. However, $\beta_c$ and $\beta_m$ were significantly decreased by both drugs in the LVH group (Table 3).

3.5. Effects of enalaprilat and L-158,809 on myocardial blood flow (Table 4)

At baseline, there were no significant differences in LV myocardial blood flow or endocardial/epicardial flow ratio between the control and LVH groups. In the control group, myocardial blood flow was not significantly altered by enalaprilat injection but was decreased by L-158,809 despite the similar decreases in systemic blood pressure between the two drugs. In contrast, both enalaprilat and L-158,809 caused significant decreases of myocardial blood flow in the LVH groups. Endocardial/epicardial flow ratio was not significantly changed by the drugs in either group.

3.6. Effects of enalaprilat and L-158,809 on plasma angiotensin II and norepinephrine

Baseline plasma concentration of angiotensin II was higher in the LVH group than in the control group (147 ±

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**Table 3**

<table>
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<tr>
<th></th>
<th>CTL-E</th>
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<td></td>
<td>Baseline</td>
<td>Enalaprilat</td>
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<td>L-158,809</td>
<td>Baseline</td>
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<tr>
<td>$\beta_c$ (mm$^{-1}$)</td>
<td>0.093 ± 0.007</td>
<td>0.095 ± 0.008</td>
<td>0.153 ± 0.010</td>
<td>0.121 ± 0.009</td>
<td>0.089 ± 0.008</td>
<td>0.082 ± 0.007</td>
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<td>$\beta_m$</td>
<td>5.31 ± 0.59</td>
<td>5.70 ± 0.41</td>
<td>9.39 ± 1.15</td>
<td>7.22 ± 0.75</td>
<td>5.49 ± 0.76</td>
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Values are expressed as mean ± s.e.m. Abbreviations are the same as in Table 1 and Table 2.

* $P < 0.05$ and ** $P < 0.01$ before and after the drugs.

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**Table 4**

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<th></th>
<th>CTL-E</th>
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<td>Enalaprilat</td>
<td>Baseline</td>
<td>L-158,809</td>
<td>Baseline</td>
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<tr>
<td>Endocardial blood flow (ml/min/g)</td>
<td>0.93 ± 0.12</td>
<td>0.85 ± 0.16</td>
<td>0.90 ± 0.12</td>
<td>0.60 ± 0.11</td>
<td>0.90 ± 0.13</td>
<td>0.68 ± 0.11</td>
<td>0.94 ± 0.13</td>
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<tr>
<td>Epicardial blood flow (ml/min/g)</td>
<td>0.77 ± 0.09</td>
<td>0.78 ± 0.11</td>
<td>0.94 ± 0.16</td>
<td>0.62 ± 0.12</td>
<td>0.79 ± 0.13</td>
<td>0.63 ± 0.10</td>
<td>0.83 ± 0.10</td>
</tr>
<tr>
<td>Endo/Epi flow ratio</td>
<td>1.20 ± 0.07</td>
<td>1.10 ± 0.11</td>
<td>1.00 ± 0.07</td>
<td>1.00 ± 0.12</td>
<td>1.18 ± 0.06</td>
<td>1.09 ± 0.06</td>
<td>1.13 ± 0.04</td>
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Values are expressed as mean ± s.e.m.

* $P < 0.05$ before and after the drug.
LV chamber and myocardial stiffness constants were significantly greater in the LVH groups (Table 1). This finding indicates alterations of LV intrinsic diastolic properties and is consonant with results of previous studies [5,6,14,22]. Intrinsic diastolic properties of the left ventricle are mainly determined by passive and active elasticity of the ventricular wall [12]. Passive elasticity is affected by structural alterations of myocardium and interstitium. On the other hand, active elasticity affects diastolic myocardial tone and is considered to be related to residual cross-bridge activation throughout diastole that results from ‘incomplete relaxation’ [12] or ‘impaired extent of relaxation’ [13] of the myocardium. The importance of active elasticity as a cause of abnormal diastolic properties has been well recognized in experimental models of demand ischemia [12]. Thus, when myocardial relaxation is incomplete and residual cross-bridge formation persists, the late diastolic pressure-volume relation is not purely passive and LV diastolic properties can be affected by active elasticity as well.

Previously, it was reported that reactive fibrosis and collagen remodeling occur and are accompanied by an increase in myocardial stiffness in the heart with pressure-overload LVH and that these histologic and functional abnormalities were improved by chronic, long-term ACE inhibitor treatment, which suggests that the renin–angiotensin system is one of the important factors to develop remodeling of the hypertrophied heart [2]. However, this mechanism cannot fully account for the present findings in which LV stiffness was improved by acute administration of enalaprilat and L-158,809. Indeed, it is known that myocyte growth and collagen accumulation are sometimes asynchronous, particularly during the early phase of evolution of LV hypertrophy [16,17]. Douglas and Tallant [16] reported that myocardial fiber diameter was increased by about 35%, but the extent of fibrosis was not significantly increased in the left ventricular wall at 12 weeks of perinephritic hypertension. Gelpi et al. [17] also found no substantial increases in connective tissue in the canine left ventricle of perinephritic hypertention (up to 14 weeks).

These observations suggest that, in addition to the alteration of passive elasticity due to structural remodeling, functional and neurohumoral factors may directly affect the active elasticity of diastolic properties in the hypertrophied left ventricle.

The effects of angiotensin II on myocardial function are considered to be related to its action on intracellular calcium handling, which seems to be mediated by the AT1-receptor and phosphoinositide second-messenger signaling pathway [7–9]. Angiotensin II is known to increase inward calcium current [7–9], to promote calcium release via phosphatidylinositol turnover [23] and to possibly cause a retardation of calcium uptake by intracellular organelles [24]. In addition, angiotensin II stimulates Na⁺-H⁺ an-

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Fig. 3. An example of LV end-diastolic (ED) pressure–diameter relations at baseline and after administration of L-158,809 in a dog with LV hypertrophy. L-158,809 decreased LV chamber stiffness constant ($\beta_c$) from 0.158 to 0.109 mmHg. The ED compliance curve also shifted downwards (arrow), reflecting an improvement in LV diastolic distensibility.

53 vs. 67 ± 16 pg/ml, $P < 0.05$). However, in the LVH group, there was a considerable scattering of the individual values of angiotensin II from the normal range to about a 10-fold increase. Nine LVH dogs showed a substantial increase in plasma angiotensin II levels above the normal range (defined as mean ± 2s.d. of the control dogs) whereas plasma angiotensin II was within the normal range in the other 11 dogs. There was no significant difference in systolic blood pressure between the 9 dogs with high angiotensin II (167 ± 5 mmHg) and the 11 dogs with normal angiotensin II (175 ± 7 mmHg, $P = NS$). After drug injection, plasma angiotensin II levels were decreased by $-123 ± 52$ pg/ml ($P < 0.05$) in the LVH-E group whereas they were increased by $122 ± 41$ pg/ml ($P < 0.05$) in the LVH-L group. In the control group, angiotensin II levels tended to increase by $81 ± 61$ pg/ml ($P = NS$) after L-158,809 injection but did not change significantly after enalaprilat injection. In the LVH group, the baseline plasma angiotensin II levels did not correlate with LVEDP ($r = -0.05$), $\beta_c$ ($r = -0.05$) or $\beta_m$ ($r = 0.02$, all $P = NS$). Further, in the LVH-E group, the changes in plasma angiotensin II levels by enalaprilat did not correlate with the changes in LVEDP ($r = -0.09$), $\beta_c$ ($r = -0.30$) or $\beta_m$ ($r = 0.03$, all $P = NS$). It is notable that LV stiffness constants were increased at baseline and were improved by enalaprilat even in the LVH dogs with normal plasma angiotensin II levels.

Baseline plasma norepinephrine levels were not significantly different between the control and LVH groups (162 ± 31 vs. 120 ± 24 pg/ml, $P = NS$). After the drug injection, plasma norepinephrine levels were decreased by $-51 ± 19$ pg/ml ($P < 0.05$) in the LVH-E group and also by $-50 ± 18$ pg/ml ($P < 0.05$) in the LVH-L group. In the control groups, however, there was no significant change in plasma norepinephrine by either drug.
tiport and induces intracellular alkalosis, thereby increasing myofilament calcium sensitivity [25]. These effects of angiotensin II on myoplasmic calcium are considered to mediate positive inotropic and chronotropic effects in the normal myocardium [7,8]. However, these effects of angiotensin II seem to be rather detrimental for intracellular calcium homeostasis in the hypertrophied myocardium and thereby may exacerbate diastolic dysfunction because the pumping ability of the sarcoplasmic calcium-sequestering system is impaired [10,11]. In addition, changes in myofilament calcium responsiveness by angiotensin II may lead to an increase in residual cross-bridge formation throughout diastole [12], resulting in alterations of the active elastic component of LV diastolic properties toward a stiffer and less distensible state.

We observed that enalaprilat and L-158,809 improved LV chamber and myocardial stiffness constants in the LVH group (Table 3). An elevation of LVVEDP after volume loading was also significantly attenuated (Table 2), and diastolic chamber distensibility was increased (Figs. 2 and 3). These findings suggest that the deleterious effects of angiotensin II on diastolic function can be ameliorated by ACE inhibition and AT1-receptor blockade in pressure-overload LVH. The present results are also consonant with previous studies. It has been shown that angiotensin II impairs myocardial relaxation and elevates diastolic pressure [1] and that these effects are prevented by enalaprilat [3] as well as by an AT1-receptor antagonist [26] in experimental LVH. A more recent clinical study also has reported an improvement in LV diastolic function by enalaprilat infusion in patients with aortic stenosis [4].

In the present study, plasma angiotensin II levels were decreased by enalaprilat injection in the LVH group. However, plasma angiotensin II levels did not correlate with the parameters of diastolic function such as LVVEDP or the stiffness constants. Indeed, plasma angiotensin II levels were found to have returned to the normal range at the time of the experiment (6 weeks after kidney wrapping) in several dogs of the LVH group despite the presence of persisting hypertension and LV diastolic dysfunction. Even in these dogs, LV diastolic properties were improved by enalaprilat and L-158,809.

It is known that the cardiac renin–angiotensin system is highly activated in pressure-overload LVH even when the circulating renin–angiotensin activity returns to normal [27,28]. It was also demonstrated that cardiac ACE is diffusely distributed within the myocardium and its density is twofold increased in the left ventricle of rats with pressure-overload LVH [29]. Locally produced angiotensin II may affect calcium-mediated myocardial force during diastole. Furthermore, another study [30] reported high-density ACE binding in connective tissue as well. Given that coiled perimysial collagen fibers assist active relaxation of myocardium by releasing stored energy [2], angiotensin II also may modulate this ‘tensity’ of connective tissue. Thus, the beneficial effects of the drugs on LV diastolic function may be related to their action on the local cardiac renin–angiotensin system.

On the other hand, both enalaprilat and L-158,809 decreased LV systolic pressure and mean aortic pressure in the control and LVH groups (Table 2). Although LV systolic function was not significantly altered by either drug in our results, the hemodynamic effects of systemic arterial pressure on LV diastolic function should be taken into consideration. Previous studies [31,32] have suggested that myocardial diastolic relaxation is sensitive to loading conditions. In the present study, the reduction in afterload due to arterial vasodilation may have contributed to an improvement of diastolic LV distensibility in the LVH group. Furthermore, systemic vasodilation with enalaprilat or L-158,809 could evoke a reflex activation of circulating catecholamines, thereby contributing to improved diastolic relaxation [33]. However, this possibility seems unlikely in the present results because plasma norepinephrine levels were significantly decreased by both drugs in the LVH group.

Another mechanism for the improvement of LV diastolic function could be related to myocardial perfusion. In the hypertrophied left ventricle, hemodynamic stress is known to induce myocardial demand ischemia because of impaired coronary vasodilatory reserve due to structural and functional alterations of the coronary circulation [34]. In the present results, however, the baseline myocardial blood flow was preserved in the LVH group (Table 4) as reported by previous studies [34]. Moreover, myocardial blood flow was rather decreased by injection of enalaprilat and L-158,809 in the LVH group (Table 4), which may at least partly reflect a decrease in oxygen demand. This finding seems consonant with previous clinical studies [35,36] that reported no change or a decrease in coronary sinus blood flow after systemic ACE inhibition. Therefore, the improvement in LV diastolic function in the LVH group is unlikely to be related to myocardial perfusion. It has been reported in previous studies [37–39] that the interactions between the renin–angiotensin system and other vasoactive substances may also influence the coronary circulation. For instance, ACE inhibition inhibits degradation of bradykinin, which in turn increases the local levels of nitric oxide and/or prostacyclin [37,38], thereby contributing to coronary vasodilation. However, this seems unlikely in LVH dogs because no substantial difference was observed between enalaprilat and L-158,809 with regard to their effects on myocardial perfusion. Nevertheless, involvement of other vasoactive neurohormonal systems is still possible, including endothelin-1, oxygen free radicals and/or endothelium-derived hyperpolarizing factor, to explain the decrease in myocardial perfusion. This remains a hypothesis unevaluated by the present study and needs further elucidation.

In conclusion, the present data suggest that angiotensin II, particularly at a local level, is involved in the pathogenesis of diastolic dysfunction in pressure-overload LVH,
and also supports the concept that ACE inhibitors and AT1-receptor blockers are potentially beneficial in the treatment of the hypertrophied heart. Thus, this study provides new insights to elucidate the underlying mechanisms of diastolic dysfunction in the hypertrophied heart.

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