The RA system plays a central role in regulating blood pressure.\textsuperscript{1-3} Angiotensin II, which is the product of the RA system, increases blood pressure by inducing constriction of vascular smooth muscle, stimulating release of aldosterone, increasing reabsorption of fluids and sodium in the kidneys, and stimulating sympathetic nerves.\textsuperscript{1-4} Altered hemodynamics in animals with cardiac depression trigger the RA system, which leads to the sustained increase in angiotensin II concentrations.\textsuperscript{5} The chronic action of angiotensin II induces proliferation of vascular smooth muscle cells, hypertrophy of cardiac muscle, and formation of interstitial fibrosis.\textsuperscript{6-8} Angiotensin II–induced vascular sclerosis and cardiac fibrosis exacerbate cardiac conditions and result in a poor prognosis.\textsuperscript{9,10}

To prevent this vicious RA-mediated cycle of cardiac deterioration, ACE inhibitors are widely used as part of the treatment for animals with cardiac disease.\textsuperscript{11-13} However, the inability of some ACE inhibitors to improve cardiac conditions suggests that there are RA pathways outside the circulatory system.\textsuperscript{15-17} Indeed, pathways for the RA system have been localized to a number of organs or to tissues within a specific organ.\textsuperscript{18-20}

Although the circulating RA system relies primarily on ACE for the generation of angiotensin II, the tissue RA system is able to produce angiotensin II independently of ACE via chymase (which is a chymostatin-like serine protease) or via kallikrein in weakly acidic conditions.\textsuperscript{14,17-22} Furthermore, the conversion of angiotensin I to angiotensin II varies among species. In humans, monkeys, dogs, and hamsters, most of the angiotensin II is generated by chymase in the heart and ACE accounts for only 10% to 20% of the angiotensin II generated.\textsuperscript{15,23-26} Conversely, in rats, mice, rabbits, guinea pigs, and swine, almost 100% of angiotensin II is produced by ACE.\textsuperscript{16} In rats, the function of chymase is unique because it cleaves angiotensin I into inactive fragments.\textsuperscript{16,22}

Conversion of angiotensin via ACE or chymase has been reported in dogs but not in cats. In addition, the clinical importance of ACE inhibitors has been recognized in dogs. For example, ACE inhibitors improve the prognosis of dogs with mitral valve insufficiency and congestive heart failure.\textsuperscript{12,27} In contrast, although ACE inhibitors have been prescribed for cats with car-

**Objective**—To clarify regulation of the renin-angiotensin (RA) system in cardiac tissues by measuring angiotensin-converting enzyme (ACE) and chymase activities in cats with pressure-overload cardiac hypertrophy.

**Animals**—13 adult cats.

**Procedures**—Pressure-overload cardiac hypertrophy was induced by coarctation of the base of the ascending aorta in 6 cats, and 7 cats served as untreated control animals. Cats were examined before and 3 months and 2 years after surgery. Two years after surgery, cardiac hypertrophy was confirmed by echocardiography, and the blood pressure gradient was measured at the site of constriction. Cats were euthanized, and ACE and chymase activities were measured in cardiac tissues.

**Results**—Mean ± SD pressure gradient across the aortic constriction was 63 ± 6 mm Hg. Chymase activity predominated (75% to 85%) in the RA system of the cardiac tissues of cats. Fibrosis in the wall of the left ventricle was detected in cats with hypertrophy, and fibrosis of the papillary muscle was particularly evident.

**Conclusions and Clinical Relevance**—Chronic pressure overload on the heart of cats can activate the RA system in cardiac tissues. A local increase in angiotensin II was one of the factors that sustained myocardial remodeling. (Am J Vet Res 2008;69:343–348)
Echocardiography was performed before and 3 months and 2 years after surgery by use of an ultrasound system and ultrasonographic probe. A left ventricular short-axis view at the level of the papillary muscle was obtained. Thickness of the interventricular septum at end of diastole and end of systole, thickness of the left ventricular free wall at end of diastole and end of systole, LVIDd, and LVIDds were measured. Variables were measured at the peak of the R-wave of the ECG for the end of diastole and at the end of the T-wave for the end of systole. Fractional shortening was calculated as \[(LVIDd - LVIDds)/LVIDd \times 100\].

Measurement of blood pressure—To measure the pressure gradient across the aortic constriction, a catheter transducer was introduced via the femoral artery and advanced to the base of the aorta. Heart rate was calculated from the ECG. The ECG and arterial pressure were analyzed by use of a computer program.

Measurement of chymase and ACE activities in cardiac tissues—After the echocardiography and blood pressure measurements were obtained at 2 years after hypertrophy-inducing surgery, all cats were euthanized by administration of an overdose of pentobarbital. The heart of each cat was removed and weighed. Cardiac tissues were cut into small blocks, frozen immediately in liquid nitrogen, and stored at 

Chymase activity in the homogenate of the left ventricular tissues was determined in accordance with methods reported elsewhere. As described in another study, angiotensin II was quantified by use of a C18 silica reverse-phase column (4.6 × 250 mm) and reverse-phase HPLC. The peak that corresponded to a synthetic angiotensin II standard was integrated to calculate formation of angiotensin II. Chymase activity was defined as chymo-statin-inhibitable angiotensin II expressed as the number of milliunits per milligram, where 1 unit was equivalent to 1 µmol of angiotensin II/min at 37°C.

The ACE activities in the homogenate of the left ventricular tissues and in serum samples were determined in accordance with the method described in another study. The ACE was extracted from homogenized cardiac tissue with a detergent and then incubated with hippuryl histidyl leucine. The reaction product, hippuric acid, was isolated from the reaction mixture by use of a C18 silica reverse–phase column (1.5 × 250 mm) and HPLC. This step eliminated interference from the detergent (ie, hippuryl histidyl leucine) and unreacted reaction by-products. The ACE activity was expressed as the number of milliunits per milligram, where 1 unit was equivalent to 1 µmol of hippuric acid/min at 37°C.

Histologic examination—Cardiac specimens were fixed in neutral-buffered 10% formalin, processed routinely, and embedded in paraffin. Tissue sections were cut at a thickness of 4 to 5 µm and stained by use of routine methods with H&E or with Azan stain (to detect collagen).

Statistical analysis—Data were expressed as the mean ± SD. Values obtained at the time of the hypertrophy-inducing surgery and at various time points after surgery were compared by use of an ANOVA followed by the Tukey post hoc test. A value of P < 0.05 was considered significant.

Results

Measurements of cardiac function by use of echocardiography and hemodynamic and morphologic changes—Clinical condition of the cats was good during the 2-year period. Changes in cardiac morphologic characteristics were evaluated by use of B-mode echocardiography of the short axes at the level of the papillary muscle and by use of M-mode echocardiography (Table 1). Use of M-mode echocardiography at the level of the papillary muscle revealed a significant (P = 0.01) increase in thickness of the left ventricular free wall at end of diastole and end of systole and thickness of the interventricular septum at end of diastole and end of systole 3 months and 2 years after hypertrophy-inducing surgery (Figure 1).

The gradient for systolic blood pressure across the constriction was (mean ± SD) 63 ± 6 mm Hg. Mean systolic blood pressure proximal to the constriction (233 ± 14 mm Hg) was significantly (P = 0.005) higher.
Fibrosis was detected than mean pressure distal to the constriction (170 ± 13 mm Hg).

Heart weight, ratio of heart weight to body weight, and ratio of left ventricular weight to body weight were significantly increased in the hypertrophy group, compared with values for the clinically normal group (Table 2). No significant differences were detected for the ratio of right ventricular weight to body weight between the 2 groups.

ACE and chymase activities in left ventricular tissues—In the clinically normal group, captopril inhibited generation of angiotensin II via the ACE system by 3.7% (mean ± SD, 0.004 ± 0.004 mU/mg of protein) and chymostatin inhibited chymase activity by 74.2% (0.06 ± 0.01 mU/mg of protein). In the hypertrophy group, captopril inhibited generation of angiotensin II via the ACE system by 6.8% (0.01 ± 0.01 mU/mg of protein) and chymostatin inhibited chymase activity by 83.9% (0.09 ± 0.01 mU/mg of protein; Figure 2). Therefore, the chymase system predominated (75% to 85%) in the RA system of cardiac tissues in cats.

Histologic examination—Fibrosis was detected in the left ventricle wall of the cats with experimentally induced hypertrophy. In particular, fibrosis of the papillary muscle was especially evident (Figure 3).

**Discussion**

Cardiac hypertrophy was induced in cats by use of nylon sutures to cause aortic coarctation. Suture ligatures do not maintain arterial constriction for a prolonged period because the vascular wall adapts to the suture. Nevertheless, in the study reported here, the mean ± SD systolic arterial pressure gradient across the constriction 2 years after surgery was maintained at 63 ± 6 mm Hg.

Echocardiography to examine cardiac function and structure 3 months and 2 years after aortic coarctation revealed significant increases in thickness of the left ventricular free wall and interventricular septum, compared with the corresponding thickness before aortic coarctation. The heart is heavier in animals with experimentally induced cardiac failure than that in control animals. Our results indicated that aortic constriction was maintained for 2 years. Furthermore, long-term pressure overload caused fibrosis in the left ventricular wall, primarily around the papillary muscles. Therefore, aortic constriction was maintained in terms of hemodynamic, morphologic, and histologic changes, and cardiac hypertrophy was successfully induced in the cats of our study.

The RA system in cardiac tissues is activated during cardiac diseases (such as cardiomyopathy, cardiac hypertrophy, cardiac infarction, mitral insufficiency, and congestive heart failure) that result in increases in angiotensin II concentrations. However, the enzymes responsible for conversion of angiotensin II have remarkable interspecies variation. In the cardiovascular system of humans, monkeys, dogs, and hamsters, chymase is the dominant protease for the generation of angiotensin II, accounting for 90% in humans and monkeys, 30% to 80% in dogs, and 30% in hamsters. In contrast, generation of angiotensin II is almost completely mediated by ACE in mice, rats, rabbits, and pigs. In rats, although

### Table 1—Mean ± SE values for echocardiographic variables in 6 cats with experimentally induced cardiac hypertrophy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>3 months</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>210 ± 5</td>
<td>221 ± 18</td>
<td>195 ± 18</td>
</tr>
<tr>
<td>LVId (mm)</td>
<td>14.9 ± 0.4</td>
<td>14.3 ± 0.8</td>
<td>13.5 ± 0.6</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>5.9 ± 0.7</td>
<td>7.6 ± 0.5*</td>
<td>6.1 ± 0.6†</td>
</tr>
<tr>
<td>LVIDw (mm)</td>
<td>3.8 ± 0.3</td>
<td>4.9 ± 0.4</td>
<td>5.4 ± 0.5*</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>5.7 ± 0.5</td>
<td>7.6 ± 0.5*</td>
<td>7.9 ± 0.6*</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>3.7 ± 0.3</td>
<td>5.6 ± 0.6*</td>
<td>6.3 ± 0.5†</td>
</tr>
<tr>
<td>IVSs (mm)</td>
<td>4.4 ± 0.2</td>
<td>6.9 ± 0.4†</td>
<td>8.6 ± 0.5‡</td>
</tr>
<tr>
<td>FS (%)</td>
<td>38.5 ± 4.4</td>
<td>46.3 ± 3.5</td>
<td>54.6 ± 4.1†</td>
</tr>
</tbody>
</table>

**Within a row, value differs significantly (**P < 0.05; †P = 0.01) from value for before surgery. ‡Within a row, value differs significantly (‡P < 0.01) from value for 3 months.

HR = Heart rate. LVId = Thickness of the left ventricular free wall during diastole. LVIDd = Thickness of the left ventricular free wall during systole. IVSd = Thickness of the interventricular septum during diastole. IVSs = Thickness of the interventricular septum during systole. FS = Fractional shortening.

### Table 2—Mean ± SD values for body weight and cardiac weights of 7 control cats and 6 cats with experimentally induced cardiac hypertrophy determined at necropsy 2 years after surgery to induce aortic constriction.

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (kg)</th>
<th>HW (g)</th>
<th>LV (g)</th>
<th>RV (g)</th>
<th>HW-BW (g/kg)</th>
<th>LVID-BW (g/kg)</th>
<th>RV-BW (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.2 ± 0.7</td>
<td>12.3 ± 3.0</td>
<td>5.3 ± 1.3</td>
<td>2.2 ± 0.7</td>
<td>3.8 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>3.4 ± 0.9</td>
<td>17.5 ± 5.2*</td>
<td>7.6 ± 2.1*</td>
<td>2.4 ± 0.5</td>
<td>5.2 ± 1.4*</td>
<td>2.3 ± 0.4</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

**Within a variable, value differs significantly (**P < 0.05; †P = 0.01) from value for the control cats. BW = Body weight. HW = Heart weight. LV = Left ventricle. RV = Right ventricle. HW-BW = Ratio of heart weight to body weight. LV-BW = Ratio of left ventricular body to body weight. RV-BW = Ratio of right ventricle weight to body weight.**

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chymase is expressed in low amounts, this enzyme does not convert angiotensin I to angiotensin II; instead, it inactivates angiotensin I by proteolytic cleavage. In the study reported here, the conversion of angiotensin I to angiotensin II in the left ventricle of cats was markedly reduced by the chymase inhibitor, chymostatin, but not by an ACE inhibitor. This indicated that chymase was dominant in the RA system in cardiac tissues of cats, accounting for 75% to 84% of all the angiotensin system.

The ACE and chymase activities are increased for 28 and 56 days, respectively, after experimental induction of infarction in the left ventricle of hamsters and then subsequently return to preinduction values. The ACE and chymase activities in left ventricular tissues from dogs with experimentally induced chronic regurgitation through the mitral valve have been evaluated. After 5 to 6 months with experimentally induced mitral valve regurgitation, chymase and ACE activities are both higher in affected dogs, compared with results for healthy dogs. In the study reported here, we compared the chymase activities between cats with cardiac hypertrophy and control cats. We found that chymase activity was 1.5 times as high in cats with induced cardiac hypertrophy, compared with the activity in the control cats, which indicated sustained chymase activity in the affected cats. In addition, tissue ACE activity was 1.7 times as high in the cats with induced hypertrophy, compared with activity in the control cats. Therefore, in cats with cardiac hypertrophy, the RA system in cardiac tissues was enhanced via both the ACE- and chymase-dependent pathways.

Renal failure and hypertrophic cardiomyopathy are common conditions in cats. Renal failure–associated hypertension, together with acute-phase reductions in renal blood flow, causes an increase in pressure overload on the heart. During the early stages of cardiac hypertrophy, hemodynamic changes cause a reduction in the systemic blood circulation, which leads to reduced renal blood flow. As a result, juxtaglomerular renin secretion is stimulated and the plasma concentration of angiotensin II increases via the action of plasma ACE in the circulating RA system. Plasma angiotensin II constricts cardiovascular smooth muscles and maintains a hypertensive state. In the chronic phase of cardiac hypertrophy, activity of the circulating RA system decreases gradually, but the sustained cardiac afterload stimulates cardiac muscle tissues and activates the RA system in cardiac tissues.

The generated angiotensin II induces myocardial hypertrophy while activating fibroblasts, which in turn leads to expansion of the extracellular matrix and myocardial fibrosis. Although we did not evaluate the circulatory RA system, ACE and chymase activities increased in cardiac tissues of cats with cardiac hypertrophy. This increase in the RA system in cardiac tissues suggested that it was the cause of the induction of cardiac hypertrophy and myocardial fibrosis.

Chymase was predominant in the cardiac tissues of cats. Chronic pressure overload on the heart of cats activated the RA system in cardiac tissues, which involved ACE and chymase, and a local increase in angiotensin II may have been one of the factors that caused sustained myocardial remodeling. This suggested that the tissue RA system can play an important role in cardiac remodeling in cats.

References


