Hypertrophic response to hemodynamic overload: role of load vs. renin-angiotensin system activation

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To distinguish between these two hypotheses, it is necessary to differentiate between the effects of an isolated change in load and the effects of RAS activation on the hypertrophic response to hemodynamic overloading in an in vivo setting. Previous in vivo studies attempting to examine the role of the ANG II receptor and the RAS in the hypertrophic response have been difficult to design and interpret for three reasons (1, 6–10, 14, 18, 20, 23, 26–28, 33, 38, 39, 42, 43). First, most pharmacological interventions designed to modulate the RAS simultaneously alter load. Even when some aspects of load were kept relatively constant, others were either not examined or were not kept constant (1, 6–10, 14, 18, 20, 23, 26–28, 33, 38, 39, 42, 43). Second, many pharmacological interventions cause secondary changes in other neurohumoral mediators, such as the increase in bradykinin that accompanies treatment with angiotensin-converting enzyme (ACE) inhibitors (13, 19, 21, 41). Third, many of the experimental methods used to chronically alter hemodynamic load and thereby mimic pertinent clinical disease states also cause primary or secondary activation of the sympathetic nervous system or the RAS (7, 16, 26, 27).
It is clear that stimulation of cardiocyte ANG II receptors can increase cardiocyte and cardiac mass (6, 22, 31, 32). In addition, the growth response to hemodynamic overload can be modified by RAS activation. Finally, it is true, at least in vitro, that hemodynamic overload produced by stretch, as one example, can activate the tissue RAS and induce cardiocytes to produce ANG II (15, 32, 40). However, the question to be addressed in this study is whether hemodynamic load can increase mass without acting through an obligatory signaling pathway involving the RAS. The current study is neither the first to ask this question, nor is it the first to support one of the two hypotheses stated above. However, as discussed below, the model used in this study offers significant advantages and confirms recently published studies (23, 35, 38, 42, 43).

An ideal in vivo experimental model in which to answer this question would 1) produce a hemodynamic overload without activation of the sympathetic nervous system or the RAS and 2) allow pharmacological modulation of the RAS without changing the extent of the hemodynamic overload used to induce cardiac hypertrophy. The current study will demonstrate that right ventricular (RV) pressure overload produced by pulmonary artery banding (PAB) in a feline model does not cause activation of either the sympathetic nervous system or the systemic RAS. This is true despite the fact that PAB results in myocardial failure. In addition, in this in vivo model, treatment with pharmacological agents that block the ANG II receptor or agents that inhibit the ACE do not alter ventricular load. This model, therefore, will allow us to more carefully examine the effect of an isolated change in load on the hypertrophic response to hemodynamic overloading.

METHODS

Study design. Four groups of cats were studied: 1) a group of 14 normal controls, 2) a group of 20 PAB cats, 3) a group of 7 PAB cats that received losartan, and 4) a group of 8 PAB cats that received captopril. The three groups of banded cats were banded identically and then studied 4 wk later.

Losartan. Losartan was administered subcutaneously through a multiperforated chronically implanted catheter. Losartan was begun 1 day before PAB and continued for 4 wk. At that time of final study, RV hemodynamics were measured. A dose-response curve of ANG II vs. systemic blood pressure was then determined. In the losartan group, the timing of the procedure was adjusted so that it fell just before the next expected dose of losartan and thus represented the nadir of AT1 blockade. Animals were included in the losartan group only if they achieved at least a one-log order increase in blood pressure ED50 (Fig. 1). One of five cats that received 15 mg losartan subcutaneously twice a day met this criteria, whereas three of five cats treated with 30 mg losartan twice a day met the criteria and three of three cats administered 60 mg losartan three times a day met this criteria. After the ANG II challenge, hearts were excised, and the papillary muscles were harvested to study muscle mechanics. The right ventricles were then compared using histological studies. With blockade of the AT1 receptor, the effects of ANG II were blocked regardless of whether ANG II was produced by the ACE pathway or the chymase pathway.

Captopril. Some studies have suggested that ACE inhibition (ACEI) acts by decreasing ANG II, whereas others suggest that ACEI acts by increasing bradykinin. Therefore, to further test the hypothesis proposed in this study, we chose to modulate the RAS proximal to the AT1 receptor by blocking ACE. Captopril was administered subcutaneously by surgically implanting a 150 mg, 60-day time-release pellet (Innovative Research of America, Sarasota, FL). This dose and formulation have been shown to be effective in providing systemic levels that completely block the ACE. Furthermore, ANG II levels were measured and demonstrate a marked decrease in ANG II in cats treated with captopril.

The studies with losartan were performed first. They showed a very consistent relation between hypertrophy at the organ level and at the cardiocyte level, i.e., the extent of increase in RV-to-body weight ratio was parallel to the increase in cardiocyte cross-sectional area. In addition, the presence of RV hypertrophy (RVH) always was associated with an abnormal papillary muscle contractile state. Because of these consistent findings and because the central goal was to determine the effect of ACEI on RVH, studies in captopril cats were limited to in vivo documentation of load and autopsy determination of RV mass.

PAB. RV pressure overload hypertrophy was induced by partially occluding the pulmonary artery with a 2.9-mm ID band, as we have described before (15, 16). Twenty cats that underwent PAB and were allowed to recover for 4 wk are presented in this study.

Effects of PAB on circulating neurohormones. Previous studies examined the effects of PAB on activation of the sympathetic nervous system and showed that PAB does not cause sympathetic activation. In the current study, activation of the systemic RAS was examined by measuring plasma levels of ANG II in cats before PAB and 4 wk after PAB in all three PAB groups. Cats were fasted overnight, and all samples were drawn at 9:00 AM before assessment of hemodynamic status. Plasma ANG II levels were determined from these samples using a quantitative competitive protein binding radioimmunoassay (Nichols Institute Diagnostics BV, Lijnden, The Netherlands).

Effects of PAB on the myocardial RAS. In five normal and five PAB cats, RV free wall and left ventricular (LV) free wall tissues were frozen in liquid nitrogen and subsequently

![Fig. 1. Dose-response curve examining effects of ANG II on change in blood pressure. Normal cats were compared with cats that had undergone pulmonary artery banding (PAB) for 4 wk and PAB cats that had received concomitant and continuous AT1 blockade with losartan. Losartan increased ED50 for increase in blood pressure by 1 log order.](image-url)
analyzed for ANG I, ANG II, and ACE activity. ANG I and ANG II concentrations were determined using previously published techniques that combine solid-phase extraction, HPLC, and radioimmunoassay (7). ACE activity was determined using previously published techniques that used hippuric acid as a substrate and quantitated the product hippuric acid by ultraviolet detection (7). ACE activity was reported as milliliters per gram tissue or milliliters per gram protein.

Hemodynamic status. At the time of final study, right heart pressure, arterial pressure, and cardiac output were determined (15, 16).

In the losartan cats, after baseline hemodynamic values were determined, each animal was challenged with a graded infusion of intravenous ANG II and the response in blood pressure was determined. ANG II was given as a bolus infusion of 0.003, 0.01, 0.1, 0.3, 1.0, and 3.0 mg. Typically, blood pressure rose in response to the ANG II infusion, reached a plateau, and then returned to baseline over a 5-min time period. After these studies, papillary muscles were removed for mechanical study, the RV and LV mass were determined, and tissues were fixed for histological examination.

In the captopril cats, after hemodynamic values were determined, the RV and LV mass were measured.

Papillary muscle isolation. In losartan cats, after assessment of hemodynamic status, RV papillary muscles were isolated and placed vertically in a 250-ml acrylic isolated muscle chamber containing Krebs-Henseleit buffer bubbled with a mixture of 95% O2-5% CO2.

The muscle was stimulated by parallel platinum electrodes delivering 5-ms pulses at a voltage of 10% over threshold. The upper end of the papillary muscle was attached to a Cambridge 300 B Servo Control System and the lower end to a semiconductor strain gauge transducer (DSC-3, Kistler-Morse). A digital computer with an analog-to-digital interface controlled either tension or length of the preparation. Tension and length data were sampled at a rate of 1 kHz and stored for later analysis. The precision of the force and length settings was 5 mg and 2 mm, respectively. The system stepped response to a length step was 95% complete in 2 ms. Equipment compliance was ~0.7 mm/mN.

The papillary muscle was then allowed to equilibrate by contracting isotonically at a light load for a period of 90–120 min. During this equilibration period, the muscle was gradually stretched to the peak of the active tension vs. length curve (Lmax defined as the muscle length resulting in peak active tension), and an isotonic contraction at 0.5-g preload and an isometric contraction at 1.5-g preload were performed. The papillary muscle was determined to be at mechanical equilibrium when values for Lmax isotonic contraction, and isometric contraction were constant over a 45-min time period. Once mechanical equilibrium was achieved, several determinations of Lmax were made. Isotonic, isometric, and graded physiologically sequenced contractions were then performed. All studies were done at 29°C. The following parameters were determined: resting and active tension (mN/mm²), peak rate of isometric tension development and tension decline (mN·mm⁻²·s⁻¹), peak rate of muscle shortening and muscle lengthening (muscle length/Δ), shortening extent (muscle lengths), time to peak tension (ms), and time to 50% relaxation (ms). Muscle length was expressed as a percentage of the resting length at Lmax (muscle lengths), and muscle load was expressed per cross-sectional area of the muscle (mN/mm²). At the conclusion of each experiment, muscle length was measured at Lmax preload. Muscle cross-sectional area was determined from muscle length at Lmax and the dry muscle weight assuming a uniform cross section, a wet weight-to-dry weight ratio of 4:1, and a specific gravity of 1.0. Muscles with cross-sectional areas <0.05 or >1.5 mm² were excluded from analysis. There were no significant differences in cross-sectional area between the three groups (in mm²: 1.0 ± 0.2 in normal, 1.2 ± 0.3 in PAB, 1.2 ± 0.2 in PAB plus losartan) and no significant differences in papillary muscle length (6.0 ± 1 in normal, 6.2 ± 1 in PAB, 6.4 ± 1 in PAB plus losartan).

Histomorphometry. In losartan cats, once the papillary muscles were excised, the RV free wall was closed with sutures. The infusion of cardioplegic solution was discontinued and replaced by an infusion of Locke’s solution containing NaCl, KCl, NaHCO3, CaCl2, and procainamide, pH 7.4. Locke’s solution was administered at a perfusion pressure of 120 mmHg for 15 min. The heart was then perfused with a fixative solution containing 1.5% glutaraldehyde, 0.2% paraformaldehyde, 0.1 M sodium cacodylate buffer, and 0.03 M CaCl2, at pH 7.4 and 300 mosM. The fixative solution was administered at a perfusion pressure of 120 mmHg for 15 min. Myocardial specimens were dissected from the RV free wall with reference to cardiocyte polarity so that accurate cross sections could be obtained. They were then placed in a postfixation buffer containing 0.1 M sodium cacodylate and 3% sucrose at pH 7.4 and 300 mosM. Tissue samples were embedded in JB4 methacrylate plastic. Two-micrometer sections were then stained with methenamine silver. Cardiocyte cross-sectional areas were found using a custom computerized image analysis system that identified cardiocyte boundaries and calculated the areas of the operator-selected cardiocytes. Thirty cell profiles per specimen were measured at the level of the nucleus at a magnification of ×1,600.

Statistical analysis. Differences between group means were determined using a multiway ANOVA and a Newman-Keuls multiple-sample comparison test. Differences were considered significant if P < 0.05. Data are presented as means ± SE.

RESULTS

In vivo hemodynamic studies. The effects of PAB, losartan, and captopril on the in vivo measurements of pressure, oximetry, and mass are summarized in Table 1 and Fig. 2. The data for normal and PAB cats were similar to those in our previous studies (3, 4). PAB caused a significant increase in RV systolic pressure and a significant increase in RV mass. In PAB animals, treatment with losartan or captopril did not alter RV loading conditions in either systole or diastole. RV systolic pressure in all three groups of PAB cats was equally increased. In addition, treatment with losartan or captopril did not alter LV loading conditions. LV pressure was unchanged by PAB, losartan, or captopril. These hemodynamic findings together with the myocardial function data below demonstrate the uniqueness of this experimental model. PAB caused RV pressure overload hypertrophy without altering LV loading conditions. Neither AT1-receptor blockade nor ACEI themselves modified the changes in RV load produced by PAB.

In PAB animals, treatment with losartan or captopril did not alter the hypertrophic response to RV pressure overload. RV mass was nearly identical in all three groups of PAB cats. Neither AT1-receptor blockade nor ACEI modified the hypertrophic response to PAB.
Morphometric studies. The effects of PAB alone and PAB plus losartan on myocyte size are summarized in Fig. 3. PAB alone caused a significant increase in myocyte surface area that was nearly identical to that found in PAB cats treated with losartan. Thus, taken together with the RV mass data above, these data indicate that at both the organ level and the cellular level, neither AT1 receptor blockade nor ACE inhibition modified the hypertrophic response to PAB.

Systemic ANG II levels. PAB did not activate the systemic RAS. ANG II levels in normal cats (66 ± 15 pg/ml) were unchanged by PAB (58 ± 17 pg/ml). Treatment with captopril caused the expected and significant reduction in ANG II levels to 8 ± 5 pg/ml. These data indicate a significant inhibition of the ACE. Losartan caused the expected and significant increase in ANG II levels to 160 ± 23 pg/ml. These data indicate a significant inhibition of the AT1 receptor.

Tissue RAS levels. ANG I, ANG II, and ACE activity were measured in RV and LV tissue from normal and PAB cats. ANG I, ANG II, and ACE activity all increased in the RV of PAB cats compared with the LV of PAB cats and with the LV or RV of normal cats. Angiotensin I was 103 ± 7 pg/g tissue in RV PAB, 56 ± 1 pg/g tissue in RV normal, 48 ± 5 pg/g tissue in LV PAB, and 47 ± 9 pg/g tissue in LV normal. ANG II was 60 ± 15 pg/g tissue in RV PAB, 37 ± 2 pg/g tissue in RV normal, 40 ± 8 pg/g tissue in LV PAB, and 38 ± 8 pg/g tissue in LV normal. ACE activity was 39 ± 8 μU/g protein in RV PAB, 17 ± 1 μU/g protein in RV normal, 8 ± 1 μU/g protein in LV PAB, and 9 ± 2 μU/g protein in LV normal. Thus, although RV pressure overload hypertrophy produced by PAB did not result in activation of systemic RAS, it did cause an increase in the local cardiac RAS.

In vitro myocardial function studies. The effects of PAB alone and PAB plus losartan on in vitro myocardial contractile state are summarized in Figs. 4 and 5. PAB caused a significant decrease in contractile state. The length vs. active tension relation was markedly depressed in the PAB compared with normal. The peak tension at Lmax decreased from 60 ± 3.5 mN/mm² in normal to 29 ± 2.8 mN/mm² in PAB muscles. In addition, the force-velocity relation was markedly depressed in the PAB when compared with normal. The peak isotonic shortening velocity decreased from 1.2 ± 0.07 muscle length (ML)/s in normal to 0.6 ± 0.04 ML/s in PAB muscles.

Treatment with losartan did not alter these effects of PAB on myocardial contractile state. PAB animals treated with losartan had a decrease in the length-active tension and force-velocity relations equivalent to

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Table 1. In vivo hemodynamic studies: pressure, oximetry, and mass

<table>
<thead>
<tr>
<th>Pulmonary Artery Band</th>
<th>Normal</th>
<th>Control</th>
<th>Losartan</th>
<th>Captopril</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV wt/body wt, g/kg</td>
<td>0.52 ± 0.04</td>
<td>1.11 ± 0.06*</td>
<td>1.06 ± 0.06*</td>
<td>1.00 ± 0.03*</td>
</tr>
<tr>
<td>RV wt/tibial length, g/cm</td>
<td>0.11 ± 0.01</td>
<td>0.27 ± 0.02*</td>
<td>0.24 ± 0.02*</td>
<td>0.25 ± 0.03*</td>
</tr>
<tr>
<td>LV wt/body wt, g/kg</td>
<td>2.2 ± 0.22</td>
<td>2.6 ± 0.15</td>
<td>2.5 ± 0.18</td>
<td>2.4 ± 0.15</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Liver wt/body wt, g/kg</td>
<td>30.5 ± 3.1</td>
<td>35.7 ± 1.6</td>
<td>34.2 ± 1.5</td>
<td>35.1 ± 1.8</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>125 ± 8</td>
<td>120 ± 7</td>
<td>130 ± 8</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>RV systolic pressure, mmHg</td>
<td>21 ± 3</td>
<td>68 ± 4*</td>
<td>65 ± 5*</td>
<td>62 ± 3*</td>
</tr>
<tr>
<td>RV diastolic pressure, mmHg</td>
<td>2 ± 2</td>
<td>5 ± 2</td>
<td>3 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Arteriovenous O2 difference, ml/l</td>
<td>37.7 ± 5.2</td>
<td>38.4 ± 2.4</td>
<td>35.2 ± 3.2</td>
<td>37.3 ± 3.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. LV, left ventricular; RV, right ventricular.

*P < 0.05 vs. normal.
that found in PAB animals. In the PAB cats treated with losartan, the peak active tension at L_{max} was 31 ± 3 mN/mm², and peak isotonic shortening velocity was 0.58 ± 0.02 ML/s.

The effects of PAB alone and PAB plus losartan on in vitro myocardial diastolic stiffness are summarized in Fig. 6. PAB caused a small but significant increase in myocardial diastolic stiffness. The passive length vs. tension relationship moved upward in the PAB compared with normal muscles. Treatment with losartan did not alter these effects of PAB on myocardial diastolic stiffness. PAB animals treated with losartan had a similar increase in the passive length vs. tension relationship.

Thus neither AT1-receptor blockade nor ACEI modified the decrease in contractile state or increase in diastolic stiffness that occurred in response to RV pressure overload hypertrophy.

**DISCUSSION**

The purpose of this study was to test the hypothesis that load, independent of the RAS, is sufficient to stimulate cardiac growth and that neither stimulation of the ANG II receptor nor RAS activation is an obligate requirement. The results of this study support this hypothesis. PAB produced RV pressure overload hypertrophy without activation of the sympathetic nervous system (2, 30) or the systemic RAS. This model of PAB allowed pharmacological modulation of the RAS with an AT1-receptor blocker, losartan, and an ACE inhibitor, captopril, without changing the extent of the hemodynamic overload induced by PAB. This model, therefore, allowed us to examine the effect of an isolated change in load on the hypertrophic response to hemodynamic overloading. Blockade of the AT1 receptor and the ACE did not alter the extent of RVH produced by PAB. Whether or not losartan was administered, the extent of the increase in RV weight, and the increase in ventricular myocyte size in PAB cats were identical. Similar results were found with captopril. Because losartan and captopril did not alter the hypertrophic response to PAB, it can be concluded that neither RAS activation nor ANG II receptor stimulation is an obligatory and necessary component of the signaling pathway that acts as an intermediary coupling load to the hypertrophic response.

It seems clear that cardiac growth can be modulated by changes either in myocardial load or neurohumoral activation. Both in vitro and in vivo studies have shown that RAS activation or AT1 stimulation is one mechanism that can regulate cardiocyte and cardiac growth (6, 22, 31, 32, 40). In studies using primary cell culture, addition of ANG II to quiescent cardiocytes caused an increase in protein synthesis rate and cardiocyte growth (22, 31, 32, 40). This growth response was blocked by AT1-receptor antagonists. Similarly, treating intact animals in vivo with ANG II results in cardiac growth (6). Again, this growth response was prevented by...
simultaneous treatment with an AT_1 antagonist (6). Alternatively, both in vitro and in vivo studies have shown that hemodynamic load can regulate cardiocyte and cardiac growth. In studies using primary cell culture, passive stretch and electrical stimulation result in an increase in protein synthesis rate and cardiocyte growth (15, 32, 37, 40). Similarly, creating hemodynamic overload in vivo results in cardiac growth. The major point of controversy lies in the interaction and proposed interdependence of load and RAS activation or AT_1-receptor stimulation. Studies using neonatal cardiocytes showed that the growth response to stretch or electrically stimulated contraction could be blocked by AT_1-receptor blockade, suggesting that RAS activation or ANG II receptor stimulation is an obligatory and necessary component of the signaling pathway that acts as an intermediary coupling load to the hypertrophic response (32). In contrast, similar studies performed in adult cardiocytes showed that the hypertrophic response to passive stretch or electrically stimulated contraction was not blocked by AT_1-receptor blockade (15).

In vivo studies of hemodynamic overload in which animals were simultaneously treated with pharmacological agents that modulate the RAS also yielded conflicting results (1, 6–9, 14, 18–20, 23, 26–28, 33, 35, 38, 39, 42, 43). The current study is concordant with those studies that showed simultaneous RAS blockade during hemodynamic overloading did not inhibit the hypertrophic response. In contrast, other studies showed that RAS blockade reduced or inhibited the hypertrophic response to hemodynamic overloading. These differences in outcome might be predicated upon specific differences in the in vivo model or experimental methods used. These include differences in banding site, the type of RAS modulation used, differences in the extent of RAS blockade, and the difficulty in being certain that RAS manipulation did not chronically alter load.

Bandling site. Differences in banding site might result in variations in the amount of RAS activation. For example, models in which LV pressure was increased by distal aortic banding close to the renal arteries may cause especially intense activation of the renal RAS, thus making it more dominant in controlling myocardial growth and more susceptible to RAS blockade. Unfortunately, however, banding site does not fully explain the differences found. Although three of four abdominal aortic-banded models did respond to RAS inhibition by blunting the hypertrophic response even when load was apparently unchanged (8, 18, 19), another apparently identical study failed to confirm these results (23). When more proximal banding techniques were used, the results remained variable. Of five studies employing proximal (aortic arch, ascending aortic or pulmonary artery) banding, three showed no effect of RAS blockade on the extent of LV or RVH (38, 42, 43), whereas in two, RAS blockade did reduce or prevent hypertrophy (28, 39). Of interest, in both the current study and the only other RV pressure overload model employing RAS manipulation that we were able to find (42), RAS blockade did not blunt the hypertrophic response. Therefore, differences in the banding site alone cannot explain the variable in vivo responses to RAS blockade. A more likely explanation for the wide differences found among the studies cited regarding the effect of the RAS on the extent of hypertrophy is the extent of activation of the RAS in the model employed. If, in a given model, the RAS is the dominant cause of ventricular hypertrophy, RAS blockade might not blunt any RAS-dependent component of the hypertrophic response. On the other hand, it is likely that when banding causes a direct LV or RV pressure overload with little RAS activation, hypertrophy develops even when the RAS is blocked. Thus, RAS blockade did not blunt hypertrophy; it was ineffective in three studies (23, 42, 43). The current study is concordant with these findings, as RAS inhibition by converting enzyme inhibitors was effective in blocking hypertrophy in three studies (8, 23, 38) but was ineffective in two, including the present study (38). When ACE inhibitors were employed, they were effective in blocking hypertrophy in three studies (18, 19, 39) and ineffective in three studies (23, 42, 43).
unrecognized differences in myocardial loading conditions that occurred simultaneously and concurrently with RAS blockade. To differentiate between the effects of RAS blockade and load, only one of these two variables must be changed while the other is held constant. Changes in cardiac load during either diastole (preload) or systole (afterload) may play a role in the regulation of cardiac growth. With the use of the law of Laplace, load or stress is determined by four factors: pressure, volume (or radius), mass (or wall thickness), and geometry. Wall stress correlates better with the extent and development of hypertrophy than pressure alone (11). Unfortunately, in banding models of hemodynamic overload, pressure must be obtained proximal to the band, which requires catheterization under anesthesia rather than the ambulatory state. In addition, measurements of volume, radius, and wall thickness may not be easily obtained. Finally, variation in geometry either caused by hemodynamic load or intrinsic to the chamber itself makes assessment of stress difficult. For example, in the studies of Weinberg et al. (38) and Litwin et al. (20), treatment with the ACE inhibitor fosinopril and the AT₁-receptor blocker BMS-186295 resulted in significant changes in systolic wall stress and diastolic wall stress. Treatment with the ACE inhibitor did not decrease the gradient across the aortic band and did not decrease LV systolic pressure but did lower end-systolic volume and in turn decreased end-systolic stress. Although this decrease in systolic stress was not likely to be the only mechanism by which the ACE inhibitor affected LV mass, its contribution cannot be ignored or excluded. Likewise, treatment with the AT₁-receptor antagonist in the same model resulted in a significant decrease in LV end-diastolic pressure and LV end-diastolic stress, which also may act to alter LV mass and alter ACE activity (7).

Despite the difficulties noted above, we believe that neither losartan nor captopril altered systolic or diastolic myocardial loading in the PAB cats for the following reasons. RV systolic and diastolic pressures were not changed by losartan or captopril. Previous studies using this model showed that RV volume is unchanged by PAB (4). The current study showed that RV mass was unchanged by losartan and captopril. Under these circumstances, RV end-diastolic volume and radius must also be unchanged unless there was a major change in diastolic myocardial compliance. The current study showed that losartan did not alter compliance of PAB papillary muscles. Thus, although RV diastolic stress was not calculated, its determinants were constant, making it likely that diastolic wall stress was constant. With respect to systole, because RV systolic pressure and RV mass were unchanged by losartan and captopril, RV end-systolic volume and radius must also be unchanged unless there was a major change in systolic contractile state. The current study showed that losartan did not alter contractile state of PAB papillary muscles. Thus, although RV systolic stress was not calculated, its determinants were constant, making it likely that systolic wall stress was constant.

Extent of RAS blockade. To fully support our conclusions, it was important to demonstrate that the pharmacological agents, the doses, and the dosing schedule used were effective in inhibiting the RAS. We struggled with this difficult issue right from the conception of this study. Two approaches were used. One approach depended on biochemically measuring changes in the concentration of specific components of the RAS. For example, an increase in ANG II resulted from treatment with an AT₁-receptor antagonist. A decrease in ANG II resulted from treatment with an ACE inhibitor. These systemic measurements were made and showed the expected results. However, there was concern that these changes in the concentration of RAS components would not ensure that the AT₁ receptor was functionally inhibited. Therefore, a second approach was used that depended on measuring the physiological response to an agonist challenge. The cardiovascular, hemodynamic, and physiological response to an ANG II infusion would prove that the AT₁ receptor was completely and effectively blocked by the AT₁-receptor antagonist. Because ANG II has little or no mechanical effect on the cardiac muscle cell itself, it is not possible to demonstrate a separate "local cardiac" functional inhibition. However, there is no question that the "cardiovascular" response to ANG II was effectively inhibited in this study. An ANG II challenge just before the next dose of losartan (when blockade would have been at its nadir) still demonstrated a 50-fold blunting of AT₁ responsiveness in terms of increasing systemic blood pressure. This fact is important and makes the current study unique because few if any previous in vivo studies have demonstrated the extent to which the RAS was blocked using functional measures. Thus RV pressure overload caused unabated hypertrophy with associated muscle dysfunction despite AT₁ blockade. Likewise, the adequacy with which captopril blocked the ACE was assessed by measuring systemic ANG II levels in each cat and demonstrating that cats treated with captopril had an eightfold fall in ANG II levels. ANG II levels would not be expected to fall to zero, since ANG II can be produced by the chymase pathway. Thus we believe we were able to achieve significant and sufficient RAS blockade in this in vivo model.

Myocardial function. AT₁-receptor blockade with losartan did not affect the development of RV systolic or diastolic papillary muscle dysfunction that is associated with this model of pressure overload hypertrophy (3, 4, 34). Whether or not losartan was administered, the reduction in contractility of papillary muscles taken from PAB cats and the increase in diastolic stiffness were identical. Thus the myocardial dysfunction that resulted from pressure overload hypertrophy occurs independent from AT₁-receptor activation.

The feline right ventricle is ideally suited for the investigation of systolic and diastolic function, since papillary muscles excised from this ventricle are small enough even when hypertrophied to avoid core ischemia. Thus the muscle bath is capable of supplying adequate nutrition and oxygenation to the papillary muscle as long as it is <1.5 mm in diameter (3), a size
typically found even in hypertrophied cat right ventricles. With the use of this model, it has been demonstrated repeatedly that PAB causes reduced force at any given muscle length and reduced contractile velocity at any given force generation compared with normal (4, 34). These findings were confirmed in this study. It might have been possible for RAS blockade to improve contractile function without blocking the extent of hypertrophy, since extent has not always correlated with the amount of hypertrophy. However, RAS blockade did not improve the systolic dysfunction that occurs in the pressure-overloaded cat right ventricle.

Conclusions. We conclude that PAB produced RV pressure overload hypertrophy without activation of the systemic RAS. Pharmacological modulation of the RAS losartan and captopril did not change the extent of the hemodynamic overload or the extent of RVH produced by PAB. Thus neither RAS activation nor ANG II receptor stimulation is an obligatory and necessary component of the signaling pathway that acts as an intermediary coupling load to the hypertrophic response. Load, in and of itself, is sufficient to stimulate cardiac growth.

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