Renin-angiotensin system and sympathetic nervous system in cardiac pressure-overload hypertrophy

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Received 10 February 2000; accepted in final form 14 July 2000

Renin-angiotensin system and sympathetic nervous system in cardiac pressure-overload hypertrophy. Am J Physiol Heart Circ Physiol 279: H2797–H2806, 2000.—Angiotensin II and norepinephrine (NE) have been implicated in the neurohumoral response to pressure overload and the development of left ventricular hypertrophy. The purpose of this study was to determine the temporal sequence for activation of the renin-angiotensin and sympathetic nervous systems in the rat after 3–60 days of pressure overload induced by aortic constriction. Initially on pressure overload, there was transient activation of the systemic renin-angiotensin system coinciding with the appearance of left ventricular hypertrophy (day 3). At day 10, there was a marked increase in AT1 receptor density in the left ventricle, increased plasma NE concentration, and elevated cardiac epinephrine content. Moreover, the inotropic response to isoproterenol was reduced in the isolated, perfused heart at 10 days of pressure overload. The affinity of the β2-adrenergic receptor in the left ventricle was decreased at 60 days. Despite these alterations, there was no decline in resting left ventricular function, β-adrenergic receptor density, or the relative distribution of β1- and β2-receptor sites in the left ventricle over 60 days of pressure overload. Thus activation of the renin-angiotensin system is an early response to pressure overload and may contribute to the initial development of cardiac hypertrophy and sympathetic activation in the compensated heart.

β-adrenergic receptor; norepinephrine; heart failure; left ventricle

Cardiac hypertrophy is an adaptive response of the heart associated with several pathological situations, including heart failure, myocardial infarction, and cardiac arrhythmias. The development of left ventricular (LV) hypertrophy enhances contractility and allows for normalization of cardiac wall stress in response to pressure or volume overload (16). The benefits of this adaptive response of the heart may be offset by detrimental effects on both cardiac function and morphology, making cardiac hypertrophy an important cause of increased morbidity and mortality. The mechanisms governing the development of cardiac hypertrophy have been extensively studied; however, they are incompletely understood. A common experimental animal model of cardiac hypertrophy is surgical aortic constriction resulting in sustained pressure overload to the heart (22). Evidence suggests involvement of neurohumoral systems such as the renin-angiotensin system and the sympathetic nervous system in the development of LV hypertrophy from cardiac pressure overload (2, 31, 39).

The sympathetic nervous system has been implicated in the development of cardiac hypertrophy, leading Ostman-Smith (34) to propose that cardiac sympathetic nerves are the final common pathway in the induction of most types of hypertrophy. In the rat aortic constriction model of cardiac pressure overload, LV norepinephrine (NE) content was decreased within 7–14 days (14, 15, 39). Reductions in cardiac NE content were generally associated with elevations in catecholamine turnover in the heart, supporting enhanced cardiac sympathetic neurotransmission (14, 15, 33, 39). Increases in cardiac sympathetic neurotransmission have been suggested to contribute to the development of hypertrophy and alterations in cardiac adrenergic receptor function. However, the majority of studies performed do not support changes in the density and/or affinity of the β-adrenergic receptor in the heart in response to pressure overload over a 1- to 4-wk period of study (7, 9, 12, 32).

Several lines of evidence suggest that the renin-angiotensin system and ANG II, produced systemically or by an intrinsic cardiac system, are activated and may contribute to cardiac hypertrophy in response to pressure overload. Components of the renin-angiotensin system, including angiotensinogen (11), angiotensin-converting enzyme (ACE) (37), and ANG II (37), are increased in the ventricle in response to pressure overload. However, disparate effects have been reported for the effectiveness of ACE inhibitors or AT1 receptor antagonists in the development of LV hypertrophy after pressure overload. Moreover, abdominal aortic constriction in AT1A receptor knockout mice produced...

Cardiac hypertrophy

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cardiac hypertrophy independent of the AT$_2$ receptor, suggesting that multiple systems are involved in the hypertrophic process, only one of which is the renin-angiotensin system (18, 19).

The intent of this study was to define the temporal sequence for neurohumoral activation in the response to cardiac pressure overload induced by abdominal aortic constriction. Definition of the status of the renin-angiotensin system and the sympathetic nervous system during the development of cardiac hypertrophy was paralleled by chronic measurement of cardiac hypertrophy and function using transthoracic echocardiography.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 275–325 g (7–9 wk of age; Harlan Sprague Dawley, Indianapolis, IN) were used in all experiments. Rats were housed two per cage with free access to food and water. All studies were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

**Surgical Induction of Pressure Overload**

Rats were randomly assigned to groups [aortic constricted (AC) and sham operated (SO)] and time points (3, 10, 30, or 60 days; $n = 8$ rats/group at each time point). Rats were anesthetized by ketamine hydrochloride plus acepromazine maleate (90 and 0.02 mg/kg ip, respectively; Fort Dodge Laboratories, Fort Dodge, IA) and prepared for surgery under aseptic conditions. After a midline abdominal laparotomy, pressure overload was induced by suprarenal abdominal aortic constriction using a tantalum Weck hemoclip (Pilling Weck, Research Triangle Park, NC) tightened to the diameter of a 22-gauge needle. Control rats underwent sham surgery consisting of midline laparotomy and isolation of the suprarenal abdominal aorta without constriction. The muscle was sutured, and the skin was closed using surgical wound clips. On the final day of the study, each rat was examined to verify the location of the hemoclip, and both kidneys were weighed to identify the presence of renal atrophy.

**Echocardiography**

LV function and chamber dimensions were assessed in a subset of rats ($n = 5$ rats/group at each time point) by transthoracic echocardiography using a diagnostic sonar ultrasound imaging system. Under ether anesthesia, chest wall hair was removed, and rats were held in the left lateral decubitus position. The ultrasound probe was positioned on the final day of study from the carotid artery catheter of the right carotid artery was cannulated with a fluid-filled polyethylene (PE-50) catheter connected to a pressure transducer in-line to a Grass polygraph (model 78D, Grass Instrument, Quincy, MA).

**Measurement of Contractility in Isolated, Perfused Heart**

The isolated, perfused heart was used to examine alterations in the cardiac inotropic responsiveness to isoprotrenol. After the administration of ketamine-acepromazine (90 and 0.02 mg/kg ip) and heparin (1,500 IU/kg), the heart was rapidly removed from the thoracic cavity by median sternotomy and immediately placed in ice-cold Krebs-Henseleit buffer (mM: 118 NaCl, 4.8 KCl, 25 NaHCO$_3$, 1.2 MgSO$_4$, 1.2 HK$_2$PO$_4$, 11 glucose, 1.5 CaCl$_2$; 5% CO$_2$-95% O$_2$; pH 7.4). Hearts were mounted onto an aortic cannula and perfused by retrograde coronary artery perfusion at constant flow with a peristaltic pump. Coronary flow rate was adjusted to achieve a baseline mean coronary perfusion pressure of 65 and 95 mmHg in SO and AC hearts, respectively. These levels of perfusion pressure were chosen based on preliminary data demonstrating an ~30-mmHg increase in the in vivo perfusion pressure in AC (10 days) compared with SO rats. Changes in perfusion pressure were continuously monitored at the level of the aortic root. To determine cardiac function, a latex fluid-filled balloon attached to PE-190 tubing was inserted into the LV. Perfusion pressure and cardiac function were obtained from in-line pressure transducers connected to a Digi-Med blood pressure analyzer and heart performance analyzer, respectively (Micro-Med, Louisville, KY). Hearts were electrically paced at 300 beats/min using two silver Teflon-coated electrodes connected to a Grass model SD9 stimulator (Grass Medical Instruments). LV balloon volume was adjusted to achieve a LV end-diastolic pressure (EDP) of 10 mmHg. After a 20-min stabilization period, baseline measurements of HR, maximal LV pressure (LVP), LVEDP, and positive first derivative of LV pressure (+dP/dt) were obtained. Subsequently, the contractile response (% change in +dP/dt) to isoproterenol (10 nM) was determined.

**Measurement of Plasma and Tissue Catecholamines**

Catecholamines were measured according to previously described methods (27). Briefly, blood (2 ml) was collected on the final day of study from the carotid artery catheter of anesthetized rats into heparin tubes and centrifuged at 1,100 $g$ for 20 min at $4^\circ$C. Plasma was stored at $-70^\circ$C until assay. A sample (50 mg) of LV free wall was frozen in liquid nitrogen. Tissue was homogenized in 0.4 N perchloric acid buffer (1 ml, containing 0.5 mM EDTA and 0.4 mM sodium metabisulphite) on ice for 10 s and centrifuged at 12,365 $g$ for 10 min at $4^\circ$C. A fixed amount (524 pg) of dihydroxybenzylamine (DHBA) was added to the supernatant of each sample as an internal standard. An aliquot (1 ml) of plasma was thawed and added to 1 ml of the perchloric acid buffer and internal standard. Catecholamines were extracted from plasma and tissue by the addition of activated alumina (25 mg; Bioanalytical Systems, West Lafayette, IN). The aluminia mixture was titrated to pH 8.7 by the addition of 3 M Tris base (pH 10.9) and vortexed for 10 min, followed by centrifugation at 3,091 $g$ for 2 min at $4^\circ$C. The supernatant was discarded, and the remaining alumina pellet was washed three times with water. Catecholamines were eluted twice by the addition of 0.15 N of perchloric acid (100 $\mu$L). Catecholamine standards [NE (50–200 pg), epinephrine (Epi; 50–200 pg), and DHBA (524 pg)] and samples were quanti-
tated by HPLC with electrochemical detection (Beckman model 116 pump and model 7725 injection valve, Rhedia, CA; Coulonex model 5100A electrochemical detector and model 5011 analytical cell, ESA, Bedford, MA). Retention times of standards were used to identify NE, Epi, and DHBA, and peak heights were used to quantify amount. The peak height was linear (correlation coefficient > 0.95) to the amount of catecholamine (NE and Epi) up to 200 pg. Extraction recovery for DHBA was >80%, and the sensitivity for catecholamines was 5 pg. All samples were diluted to give peak heights within the range of 50–200 pg and corrected for recovery and dilution.

Measurement of Plasma Angiotensin Peptides

Plasma angiotensin peptide concentration was measured according to a previously described method (6). Blood (5 ml) was collected from the carotid artery catheter of anesthetized rats on the final day of study into tubes containing 125 mM EDTA, 20 mM phenanthroline, 0.2% neomycin, 0.1 mM kalikrein, 2% ethanol, and 2% DMSO (250 μl) to eliminate both the production and breakdown of angiotensin peptides during sample handling. Angiotensin peptides from plasma (2 ml) were extracted using SepPak C-18 column chromatography. Angiotensin peptide concentration in each plasma sample was measured by RIA using a polyclonal ANG II antibody (Dr. A. Freedlender, University of Virginia, Charlottesville, VA) that exhibited minimal cross reactivity to ANG I (2%) and ANG II fragment 5–8 (4%), but 100% cross reactivity to ANG III, ANG II fragment 3–8, and ANG II fragment 4–8. Sensitivity of the RIA was 2 pg/ml.

LV Membrane Preparation

After hemodynamic measurements and blood collection for neurohumoral measurements, hearts were removed and placed in ice-cold Krebs buffer. The LV was dissected free from the atria and right ventricle to obtain absolute cardiac chamber wet weights. LV weight normalized to body weight (LV/BW) was used as an index of cardiac mass for the determination of cardiac hypertrophy. Whole LV, including the interventricular septum, was placed in 30 ml of ice-cold membrane buffer (50 mM NaPO₄, 0.25 M sucrose; pH 7.2), homogenized on ice using a Polytron for 20 s, and centrifuged at 1,100 × g for 10 min at 4°C. The resulting pellet was discarded, and the supernatant was centrifuged three times at 48,000 × g for 10 min at 4°C. The final pellet was resuspended 3 ml of buffer containing 50 mM NaPO₄, 0.1 mM EDTA, 28 kalikrein inhibitory units (KIU)/dl aprotinin, and 0.014% bacitracin; pH 7.2), homogenized, and stored at −70°C. Protein concentration was determined by the method described by Bradford (4).

β-Adrenergic Receptor Binding Assays

Binding assays (saturation isotherm, competition) for β-adrenergic receptors were performed in membranes prepared from the LV. Saturation isotherms for the β-adrenergic receptor were performed by adding an increasing concentration (3–400 pM) of (−)[125I]iodocyanopindolol ([125I]ICYP, nonselective β-adrenergic receptor antagonist; specific activity 2,200 Ci/mM, Peptide Radioiodination Service Center, Washington State University) to a fixed amount of membrane protein (75 μg) in tubes containing binding assay buffer (50 mM Tris·HCl, 0.1 mM EDTA, 1 mM MgCl₂, 28 KIU/dl aprotinin, 0.014% bacitracin, 0.2% bovine serum albumin; pH 7.2). Nonspecific binding was determined at each radioligand concentration by the addition of the nonselective β-receptor antagonist propranolol (10 μM). Incubation was performed in a total volume of 0.25 ml for 180 min at 25°C and terminated by filtration through Whatman GF/B glass-fiber filters (presoaked in 50 mM Tris·HCl buffer) using a Brandel harvester. Filters were washed three times with ice-cold binding buffer, and the amount of radioactivity retained on the filter was determined in a gamma-counter (model A550, Packard, Dowers Grove, IL). Maximal number of binding sites (Bₘₐₓ) and affinity (Kᵢ) were derived by nonlinear regression analysis using LIGAND software.

Competition studies were performed using the selective β₁-adrenergic receptor antagonist CGP-20712A. Competition was performed using a fixed concentration of [125I]ICYP (50 pM; 2.5 times Kᵢ) with a range of CGP-20712A concentrations (10⁻¹⁰–10⁻⁷ M). The inhibitory constant (Kᵢ) value for CGP-20712A at the β₁- and β₂-receptors was calculated according to the equation derived by Cheng and Prusoff (8). One- and two-site models were fit to competition binding data using LIGAND software.

AT₁ Receptor Binding Assay

Saturation isotherms for the AT₁ receptor were performed by adding a fixed amount of LV membrane protein (75 μg) to tubes containing the binding assay buffer and an increasing concentration (0.05–5 nM) of [125I]-labeled [Sar¹Ile⁸]ANG II (nonselective ANG II receptor antagonist; specific activity 2,176 Ci/mM, Peptide Radioiodination Service Center, Washington State University). The AT₁ receptor antagonist PD-123319 (1 μM) was included in the binding buffer to eliminate binding of [125I]-labeled [Sar¹Ile⁸]ANG II to the AT₂ receptor site. Nonspecific binding was determined at each radioligand concentration by the addition of an excess of unlabeled ANG II (10 μM). Incubation was performed in a total volume of 0.25 ml for 60 min at 26°C and terminated by filtration through Whatman GF/B glass-fiber filters (pre-soaked in 50 mM sodium phosphate buffer containing 1% polyethylenimine) using a Brandel harvester. Filters were washed three times with ice-cold binding buffer. Bₘₐₓ and Kᵢ were derived by nonlinear regression analysis using LIGAND software.

Statistical Analysis

Data are presented as means ± SE. For each parameter measured (hemodynamic, LV/BW, plasma NE, plasma ANG II, Bₘₐₓ, Kᵢ) in the time course study, separate two-way ANOVAs were performed for each parameter with treatment (AC, SO) and time (3, 10, 30, and 60 days) as between-group factors. Serial echocardiographic studies were performed in a subset (n = 5) of AC and SO rats at baseline and 3, 10, 30, and 60 days. A two-way ANOVA (group × time) with time as a repeated measure was performed to determine differences in wall thickness and LV function. The Student-Newman-Keuls test was used for post hoc comparisons of individual parameters across group and time. For data (Epi, NE, Kᵢ, and Bₘₐₓ) in the 10-day study, a two-tailed t-test was performed to determine differences between groups (AC, SO). P values <0.05 were considered statistically significant.

RESULTS

Time Course for Alterations in LV Hypertrophy, Cardiac Function, Sympathetic Nervous System, and Renin-Angiotensin System After Pressure Overload

Magnitude of pressure overload. The time course for alterations in blood pressure and heart rate after 3, 10,
Table 1. Hemodynamic measurements in AC and SO rats after pressure overload

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time, days</th>
<th>MAP, mmHg</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>3</td>
<td>111 ± 2</td>
<td>128 ± 3</td>
<td>97 ± 2</td>
<td>424 ± 9</td>
</tr>
<tr>
<td>AC</td>
<td>3</td>
<td>142 ± 5*</td>
<td>171 ± 8*</td>
<td>121 ± 4*</td>
<td>383 ± 20</td>
</tr>
<tr>
<td>SO</td>
<td>10</td>
<td>110 ± 3</td>
<td>124 ± 3</td>
<td>96 ± 3</td>
<td>423 ± 20</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>144 ± 7*</td>
<td>171 ± 8*</td>
<td>121 ± 6*</td>
<td>401 ± 24</td>
</tr>
<tr>
<td>SO</td>
<td>30</td>
<td>109 ± 5</td>
<td>134 ± 7</td>
<td>95 ± 3</td>
<td>399 ± 7</td>
</tr>
<tr>
<td>AC</td>
<td>30</td>
<td>141 ± 4*</td>
<td>174 ± 7*</td>
<td>116 ± 3*</td>
<td>413 ± 5</td>
</tr>
<tr>
<td>SO</td>
<td>60</td>
<td>116 ± 2</td>
<td>143 ± 2</td>
<td>101 ± 2</td>
<td>413 ± 10</td>
</tr>
<tr>
<td>AC</td>
<td>60</td>
<td>152 ± 4*</td>
<td>188 ± 8*</td>
<td>123 ± 4*</td>
<td>373 ± 11</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 8 rats/group for each time point. AC, aortic constricted; SO, sham operated; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. *P < 0.0001, AC vs. SO rats at respective time point.

30, and 60 days of pressure overload is presented in Table 1. Abdominal aortic constriction resulted in a significant increase in MAP (F1,58 = 135; P < 0.0001), SBP (F1,58 = 111; P < 0.0001), and DBP (F1,58 = 87; P < 0.0001). MAP was increased by ~32 mmHg in AC rats at each time point after pressure overload. HR was not different between AC and SO rats at each time point and within each group across time.

Body weight and atrial and ventricular weights were analyzed across group and time. There was a significant (F3,60 = 125; P < 0.0001) increase in body weight with time in rats from both groups, with no significant difference between groups (data not shown). LV hypertrophy developed by day 3 and was maintained through 60 days of pressure overload. LV hypertrophy was manifested as an increase in absolute LV weight (F1,60 = 102; P < 0.0001), which remained evident when normalized to body weight (F1,60 = 115; P < 0.0001; Fig. 1A). Left atrial hypertrophy was observed in AC rats from day 10 through day 60 (F1,60 = 41; P < 0.0001). Right ventricle and atrial weights were not different between the two groups at all time points (data not shown). Right and left kidney weights were similar in SO (right 1.29 ± 0.03, left 1.28 ± 0.03 g) and AC (right 1.17 ± 0.03, left 1.31 ± 0.03 g) rats at each point after pressure overload. Only four rats in the AC group demonstrated right renal atrophy, which occurred in three rats after 60 days of pressure overload.

Echocardiographic measurements. EDD and ESD were not different between AC and SO rats at any time point after pressure overload (data not shown). Similarly, LV FS was not different in AC and SO rats at any time point (Fig. 1B). AWT was significantly increased (F1,16 = 16; P < 0.01) in AC compared with SO rats at days 3–60. PWT was not different between AC and SO rats at any time point (data not shown).

Plasma NE and ANG II concentration. Statistical analysis of plasma NE concentration after pressure overload revealed a significant effect of group (F1,36 = 7; P < 0.05) and time (F3,36 = 6; P < 0.05). Plasma NE concentration was not different in AC and SO rats at day 3; however, plasma NE concentration was increased in AC rats by day 10 and remained elevated through day 60 (Fig. 2A). Statistical analysis of plasma ANG II concentration revealed a significant effect of time (F3,38 = 22; P < 0.0001), no between-group effect, and a significant interaction between time and group (F3,38 = 4; P < 0.05). Plasma ANG II concentration was increased by 63% in AC versus SO rats at day 3. Moreover, plasma ANG II concentration at day 3 in both groups was significantly increased compared with days 10–60 (Fig. 2B).

LV β-adrenergic receptors. Initial binding experiments using [125I]ICYP were performed in control rats to determine optimal membrane protein and time to equilibrium (data not shown). A representative saturation binding isotherm with corresponding Scatchard...
plot for specific $[^{125}\text{I}]$ICYP binding in rat LV membranes prepared from AC and SO rats after 60 days of pressure overload is illustrated in Fig. 3A. Specific binding of $[^{125}\text{I}]$ICYP was saturable and best described by a one-site model. The affinity and density for $[^{125}\text{I}]$ICYP binding were not influenced by time in either group (Table 2). Moreover, there was no effect of pressure overload on the affinity or density for $[^{125}\text{I}]$ICYP binding in LV membranes over the time course examined.

Competition of $[^{125}\text{I}]$ICYP binding with the $\beta_1$-receptor antagonist CGP-20712A in LV membranes from AC and SO rats was concentration dependent and best described by a two-site model (Fig. 3B). A high-affinity ($K_{i1}, \beta_1$) and a low-affinity ($K_{i2}, \beta_2$) site were defined by CGP-20712A competition (28). Statistical analysis revealed a significant effect of time on the $K_{i1}$ ($F_{2,12} = 7; P < 0.01$) and $K_{i2}$ ($F_{2,12} = 35; P < 0.0001$) for CGP-20712A in AC and SO rats (Table 2). Derived $K_i$ constants for the high- and the low-affinity site were significantly increased ($P < 0.05$) in AC and SO rats at day 60 of pressure overload compared with days 3 and 10. Moreover, the $K_{i2}$ value for CGP-20712A was significantly increased ($P < 0.001$) in AC compared with SO rats after 60 days of pressure overload. In LV membranes from SO rats, the relative proportion (% $\beta_1$ subtype) of $\beta_1$- and $\beta_2$-receptors averaged 54% (Table 2). The proportion of $\beta_1$- and $\beta_2$-receptors in LV was not significantly influenced by pressure overload at any time point.

Fig. 3. Saturation isotherm and CGP-20712A competition of $(-)^{[125}\text{I}]$iodocyanopindolol ($[^{125}\text{I}]$ICYP) binding in LV membranes from 60-day AC and SO rats. A: saturation isotherms were performed by adding a fixed amount of membrane protein to increasing concentrations of $[^{125}\text{I}]$ICYP. Inset, Scatchard transformation of saturation isotherm data. $[^{125}\text{I}]$ICYP bound to a single class of sites with high affinity in membranes from AC and SO rats, with no between-group differences. B: bound; B/F, bound/free. B: competition of $[^{125}\text{I}]$ICYP binding by increasing concentrations of the selective $\beta_1$-adrenergic receptor antagonist CGP-20712A. CGP-20712A displaced $[^{125}\text{I}]$ICYP in a concentration-dependent manner; competition data fit a 2-site model, with a high- and a low-affinity site for CGP-20712A displacement. Data are means ± SE ($n = 4$ rats/group).
Cardiac Renin-Angiotensin System and Sympathetic Nervous System After 10 Days of Pressure Overload

In a separate study, cardiac catecholamine content, AT1 receptor density, and cardiac function were examined at 10 days of pressure overload. This time point was chosen to determine whether increases in plasma ANG II concentration (day 3) subsequently influenced the cardiac AT1 receptor and to determine whether elevations in plasma NE concentration (day 10) were associated with alterations in cardiac catecholamine content and cardiac dysfunction. In agreement with results from the time course study, there was a significant increase in LV/BW at 10 days of pressure overload (SO 2.1 ± 0.1, AC 3.3 ± 0.1; P < 0.05). LV NE content was not significantly altered after 10 days of pressure overload (SO 635 ± 60, AC 521 ± 86 ng/g tissue). In contrast, LV Epi content was significantly increased in AC rats (SO 487 ± 96, AC 860 ± 110 ng/g tissue; P < 0.05).

Radioligand binding assays for the AT1 receptor were performed in LV membranes prepared from AC and SO rats after 10 days of pressure overload. Saturation isotherms demonstrated that specific binding of [125I]labeled [Sar1, Ile8]ANG II in LV membranes was saturable and best described by a one-site model, with no differences in binding affinity between AC (2.2 ± 0.7 nM) and SO rats (1.3 ± 0.1 nM). However, the density of AT1 receptor sites in LV was increased fivefold in AC rats after 10 days of pressure overload (SO 8.7 ± 0.8, AC 42.2 ± 9 fmol/mg protein; P < 0.05).

In a separate group of rats subjected to 10 days of pressure overload, the contractile (+dP/dt) response to a single EC50 (10 nM) of isoproterenol was determined in the isolated, perfused heart (Fig. 4). There was a significant increase in LV/BW in AC compared with SO rats (SO 2.9 ± 0.1, AC 4.2 ± 0.2; P < 0.05). There was no difference between AC and SO rats in baseline +dP/dt (SO 2,772 ± 259, AC 2,729 ± 618 mmHg/s; P > 0.05). Isoproterenol increased contractility (% increase in +dP/dt) in hearts from both SO and AC rats; however, the contractile response to isoproterenol was significantly reduced (by 35%) in AC compared with SO rats (Fig. 4).

Table 2. Left ventricular β-adrenergic receptor binding of [125I]ICYP in AC and SO rats after pressure overload

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time, days</th>
<th>Kd, pM</th>
<th>Bmax, fmol/mg protein</th>
<th>% β1, Subtype</th>
<th>Kd, nM</th>
<th>Kd, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>3</td>
<td>21.03 ± 1.39</td>
<td>83.48 ± 3.85</td>
<td>56.2 ± 3.5</td>
<td>1.25 ± 0.31</td>
<td>3.14 ± 0.36</td>
</tr>
<tr>
<td>AC</td>
<td>3</td>
<td>22.60 ± 2.30</td>
<td>82.99 ± 5.43</td>
<td>49.0 ± 2.4</td>
<td>2.01 ± 0.42</td>
<td>4.37 ± 0.56</td>
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<tr>
<td>SO</td>
<td>10</td>
<td>20.33 ± 2.23</td>
<td>82.72 ± 3.64</td>
<td>50.4 ± 5.0</td>
<td>1.87 ± 0.37</td>
<td>3.79 ± 0.06</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>19.45 ± 1.64</td>
<td>80.03 ± 2.16</td>
<td>45.2 ± 0.9</td>
<td>1.83 ± 0.58</td>
<td>4.01 ± 0.14</td>
</tr>
<tr>
<td>SO</td>
<td>60</td>
<td>23.87 ± 3.30</td>
<td>73.89 ± 2.76</td>
<td>56.7 ± 2.8</td>
<td>5.17 ± 0.81†</td>
<td>5.48 ± 0.92†</td>
</tr>
<tr>
<td>AC</td>
<td>60</td>
<td>19.84 ± 2.94</td>
<td>76.41 ± 5.20</td>
<td>51.9 ± 3.3</td>
<td>6.68 ± 0.99†</td>
<td>12.98 ± 0.29†</td>
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</table>

Data are means ± SE; n = 4 rats/group for each time point. [125I]ICYP, (−)-[125I]iodocyanopindolol; Kd, receptor affinity; Bmax, receptor density; Kd, high-affinity site for CGP-20712A at β1-receptor subtype; Ky, low-affinity site for CGP-20712A at β2-receptor subtype. *P < 0.05, AC vs. SO rat at respective time point. †P < 0.05, day 60 vs. days 3 and 10 within each group (AC, SO).

DISCUSSION

Our results demonstrate that neurohumoral activation of the renin-angiotensin system and the sympathetic nervous system are elicited in a temporally defined sequence in response to pressure overload (Fig. 5). Initially on pressure overload, there is transient activation of the systemic renin-angiotensin system coinciding with the appearance of LV hypertrophy, followed by a marked increase in AT1 receptor density in the LV, generalized increases in sympathetic nervous system activity, and a decline in the response to inotropic challenge. At 60 days of pressure overload, alterations in the affinity of the β2-adrenergic receptor site for CGP-20712A were demonstrated in the LV. Despite these initial alterations, there was no decline in resting LV function, as defined by echocardiography, the density of β-adrenergic receptor sites, or the relative distribution of β1- and β2-receptor sites in the LV over 60 days of pressure overload. Thus activation of the systemic renin-angiotensin system is an early response to aortic constriction and may initiate development of cardiac hypertrophy and sympathetic activation. In contrast, activation of the sympathetic nervous system appears to underlie the progression and main-
tenance of cardiac hypertrophy in response to pressure
load. Rapid activation of these neurohumoral sys-
tems and the development of cardiac hypertrophy re-
sult in compensated heart function at rest but rapid
decline in the responsiveness to inotropic challenge.

In general, our results are in agreement with find-
ings from previous studies examining the time course
for cardiac hypertrophy and function in response to
pressure overload. In this study, pressure overload
induced by suprarenal abdominal aortic constric-
tion increased MAP proximal to the site of aortic con-
striction. The magnitude of the pressure increase was suf-
cient to produce sustained LV and left atrial hyper-
trophy within a 3-day time frame. In agreement with
these findings, an increase in LV/BW was demonstrated
within 3 days of aortic constriction (39). In other
studies, an increase in ventricular RNA content
was detected after 3 days of aortic constriction (14);
however, LV-to-BW ratios were not significantly in-
creased until day 14, suggesting that increases in ven-
tricular protein synthesis precede the development
of cardiac hypertrophy. Echocardiographic analysis
was consistent with postmortem measurements in this
study demonstrating an increase in AWT with normal
chamber dimensions in AC rats after 3–60 days of
pressure overload. Previous investigators demon-
strated that pressure overload is initially characterized
by the development of concentric LV hypertrophy with
compensated LV contractile performance (29, 31). Our
results demonstrate that hypertrophy of the LV within
3 days of aortic constriction compensates to maintain
baseline cardiac function over an 8-wk time frame of
pressure overload.

Previous studies demonstrated that subdiaphrag-
matic aortic constriction resulted in a rapid elevation
in plasma renin activity, which returned to control
values during the chronic phase of pressure overload
(2). We measured plasma ANG II concentration after
pressure overload to determine the status of the sys-
temic renin-angiotensin system. In both groups of rats,
plasma ANG II concentration was elevated at day 3
compared with levels at days 10–60, suggesting that
the stress of surgery initially activated the renin-an-
giotensin system. However, at 3 days of pressure over-
load, plasma ANG II concentration was elevated in AC
compared with SO rats. Our results extend previous
findings by demonstrating an increase in plasma ANG
II at 3 days after suprarenal abdominal aortic constric-
tion. Plasma ANG II concentrations in AC and SO rats
beyond day 3 were not different and were in agreement
with previously published values in the rat (38). Acute
increases in plasma ANG II after suprarenal aortic
constriction in the present study are consistent with
increases in the secretion of renin from the juxtaglo-
merular cells of the kidney in response to the initial
reduction in renal perfusion pressure (17). Brilla et al.
(5) reported a normal plasma ANG II concentration in
rats subjected to infrarenal aortic constriction from 1 to
8 wk. In contrast, plasma ANG II concentration in-
creased within 1 wk and remained elevated for 8 wk
after suprarenal aortic banding with constriction of the
right renal artery and subsequent atrophy of the right
kidney. Thus our results of a transient increase in
plasma ANG II concentration after pressure overload
induced by suprarenal aortic constriction without co-
existant renal atrophy demonstrate that differences in
plasma ANG II concentration between various studies
using the pressure-overload model of aortic constric-
tion most likely result from variability in the place-
ment of the vascular constriction in relation to the
renal arteries.

In agreement with previous results (35), a modest
density of AT1 receptor sites was observed in the LV of
SO control rats. After 10 days of pressure overload, a
marked increase (5-fold) in AT1 receptor density was
observed in the LV. In an aortocaval shunt model of
volume overload with associated cardiac hypertrophy,
a marked increase in AT1 receptor density was previously
demonstrated (23). Together, these results demon-
strate an increase in cardiac AT1 receptor density in
cardiac hypertrophy resulting from volume or pressure
overload. Thus alterations in neurohumoral mediators
including systemic and/or cardiac ANG II may contri-
bute to hypertrophy independent of the hemodynamic
stress associated with elevated blood pressure. In sup-
port of this, Heller et al. (20) demonstrated a positive
correlation between plasma or LV renin concentration
and the degree of cardiac hypertrophy at 3 days of
pressure overload but not by 42 days of pressure over-
load, despite sustained elevations in systolic pressure.
In contrast to results from our study, Lopez et al. (30)
reported a reduction in cardiac AT1 receptor density

 Fig. 5. The temporal sequence for activation of the renin-angiotensin
system and the sympathetic nervous system in cardiac hypertrophy
from pressure overload. Increases in plasma ANG II occur early after
suprarenal aortic constriction and are not maintained. LV hypertro-
phy (LVH) is evident within 3 days of pressure overload, coinciding
with transient elevations in plasma ANG II. By day 10, plasma NE
concentration is elevated, AT1 receptor density and epinephrine
(Epi) content are increased in the ventricle, and LVH is maintained.
Through day 60, LVH and elevations in plasma NE are maintained,
but fractional shortening and ventricular β-receptor density are
normal. On day 60, the affinity of the β2-receptor in the ventricle is
decreased. RBF, renal blood flow.
after 8 wk of pressure overload. Moreover, in AT_{1A} receptor knockout mice subjected to abdominal aortic constriction for 2 (19) or 3 (18) wk, LV hypertrophy was unabated, demonstrating that hypertrophy can develop independent of AT_{1} receptor stimulation. We suggest that elimination of one of these neurohumoral systems, such as in the AT_{1A} receptor knockout, is compensated by other neurohumoral mediators capable of initiating LV hypertrophy and the maintenance of cardiac function. In this study, pressure overload resulted in temporally defined activation of the renin-angiotensin system and the sympathetic nervous system, despite consistently elevated systolic pressure. Moreover, our results of a marked increase in cardiac AT_{1} receptor density at 10 days of pressure overload are consistent with a direct growth-promoting effect of ANG II contributing to the early increase in cardiac mass.

In addition to the neurohumoral influences of ANG II on cardiac mass, previous investigators demonstrated significant coronary vascular and myocardial lesions as early as 1 wk after pressure overload induced by aortic constriction (36). Previous studies suggested that coronary vascular and myocardial lesions may be related to neurohumoral factors induced by aortic constriction. For example, increases in plasma ANG II concentration were suggested to contribute to cardiomyocyte necrosis and coronary vascular damage (41). In the animal model of chronic ANG II infusion, widespread multifocal areas of myocyte necrosis were observed within 2 days and were accompanied by significant cellular infiltration (24). These effects of ANG II on myocardial necrosis were prevented by the administration of the AT_{1} receptor antagonist losartan. The potential role of ANG II-mediated myocardial necrosis is consistent with findings from this study demonstrating an increase in cardiac AT_{1} receptor density early after pressure overload. Moreover, consistent with results from this study, ANG II-mediated myocyte necrosis has been specifically linked to interactions with cardiac sympathetic neurons (21).

We measured plasma NE concentration to determine the status of the sympathetic nervous system after pressure overload. Plasma NE concentration of SO rats was similar to reported values in anesthetized rats (3, 27). Siri (39) reported a progressive increase in plasma NE concentration in AC rats that reached statistical significance within 7 days. Our results extend these findings by demonstrating increased plasma NE concentration after 10 days of pressure overload that remained elevated over 60 days. Interestingly, acute elevations in circulating ANG II were demonstrated to cause increases in plasma NE concentration (10, 25, 40). In the present study, increases in plasma ANG II preceded elevated plasma NE concentration. Given the interrelationships between these two systems, these results are consistent with observations suggesting that initial increases in plasma ANG II stimulate the noradrenergic nerve terminals of the sympathetic nervous system and raise plasma NE concentration. We suggest that the time course for increases in plasma ANG II and NE support the activation of the systemic renin-angiotensin system as the initial mechanism for elevations in plasma NE, whereas increased activity in tissue renin-angiotensin systems or other neurohumoral mediators may contribute to sustained elevations in plasma NE during prolonged periods of pressure overload (11, 37).

Previous investigators demonstrated that aortic constriction increased (1–7 days) plasma Epi concentration (39). This is the first study to demonstrate elevations in cardiac Epi content after pressure overload. Cardiac Epi content is derived from adrenal-released Epi taken up from the plasma (26). Future studies must be undertaken to determine whether aortic constriction results in stimulation of the release of Epi from the adrenal medulla or, alternatively, enhanced cardiac uptake of Epi. Interestingly, evidence demonstrates that Epi can facilitate NE release from sympathetic nerve terminals through actions at presynaptic β_{2}-adrenergic receptors (1). Thus increases in cardiac Epi content after pressure overload may contribute to elevations in cardiac sympathetic neurotransmission. A limitation of the present study is the possible influence of anesthetic on plasma NE and Epi concentrations, which may differ between hypertensive and normotensive rats.

Despite sustained increases in circulating NE concentration from 10 to 60 days of pressure overload, the density of β-adrenergic receptor sites in the LV was not altered over 60 days of pressure overload. These results are in agreement with previous studies demonstrating that cardiac β-adrenergic receptor density was not altered at 3 (14) and 4 (7) wk of pressure overload. In addition, our results extend these findings by demonstrating that the relative β_{1}- to β_{2}-receptor subtype distribution was not altered after pressure overload. Despite normal β-adrenergic receptor density and subtype distribution in the LV, the response to inotropic challenge with isoproterenol was reduced in the isolated, perfused heart from rats subjected to 10 days of pressure overload. These results are in agreement with previous studies demonstrating that despite normal baseline cardiac function, inotropic responsiveness is depressed in the aortic constriction model of pressure overload (13). Thus, rather than receptor downregulation, desensitization of the cardiac β-adrenergic receptor may be an early response to elevations in systemic and cardiac sympathetic nerve activity.

An unexpected finding in this study was that the affinity of CGP-20712A for the β_{1}- and β_{2}-receptor sites in LV was shifted to a lower affinity in both groups of rats at 60 days. Moreover, the K_{D} for the β_{2}-site was significantly increased (lower affinity) in ventricle membranes from 60-day AC rats compared with controls. This may be related to the increase in cardiac Epi, which is capable of acting on the β_{2}-adrenergic receptor.

In conclusion, results from this study demonstrate that transient increases in plasma ANG II concentration precede elevations in the systemic concentration of NE after cardiac pressure overload. LV hypertrophy
was evident within 3 days of pressure overload, coincident with increases in plasma ANG II and preceding sympathetic activation. At 10 days of pressure overload, marked increases in cardiac AT1 receptor density, elevated cardiac Epi content, and impaired responsiveness to inotropic challenge were evident. All of these changes occurred in the absence of detectable alterations in cardiac β-adrenergic receptor density or declines in resting cardiac function. These results demonstrate that activation of the systemic (plasma ANG II) and cardiac (AT1 receptor density) renin-angiotensin system occur early in the development of LV hypertrophy. In contrast, the time course for sympathetic activation. At 10 days of pressure overload, coincident with increases in plasma ANG II and preceding marked increases in cardiac AT1 receptor density, elevated cardiac Epi content, and impaired responsiveness to inotropic challenge were evident. All of these changes occurred in the absence of detectable alterations in cardiac β-adrenergic receptor density or declines in resting cardiac function. These results demonstrate that activation of the systemic (plasma ANG II) and cardiac (AT1 receptor density) renin-angiotensin system occur early in the development of LV hypertrophy. In contrast, the time course for sympathetic activation may contribute to subsequent sympathetic stimulation and the continued maintenance and progression of LV hypertrophy. The technical expertise of Victoria King and Michael Fettinger contributed to the completion of these studies. This research was supported by National Institutes of Health Grant 52934 and by an internal pilot research grant on the biology of aging (Dr. P. Wise).

REFERENCES


