Load Versus Humoral Activation in the Genesis of Early Hypertensive Heart Disease

Chari Y.T. Hart, MD; Donna M. Meyer; Henry D. Tazelaar, MD; Joseph P. Grande, MD; John C. Burnett, Jr, MD; Philippe R. Housmans, MD; Margaret M. Redfield, MD

Background—The role of load versus angiotensin II (Ang II) and endothelin-1 (ET) in the pathogenesis of hypertensive heart disease is controversial. We sought to determine whether alterations in cardiac structure and function due to hypertension (HTN) were dependent on Ang II or ET activation.

Methods and Results—Bilateral renal wrapping to produce HTN (n=12) or sham surgery (n=6) was performed in adult dogs. Weekly blood pressure, plasma renin activity, Ang II, ET, and catecholamines were measured. Systolic (end-systolic elastance, Ees) and diastolic (τ) function were assessed in sham and HTN dogs at 5 (HTN-5wk) or 12 (HTN-12wk) weeks. Ang II and ET were assayed in the left ventricle (LV) and kidney. Mean arterial pressure was higher in renal wrap dogs at week 1 (*P<0.05 versus controls: 139±4* versus 123±4 mm Hg), week 5 (174±7* versus 124±4 mm Hg), and week 12 (181±12* versus 124±4 mm Hg). LV mass index was increased in HTN-5wk (22%*) and HTN-12wk (39%*). LV fibrosis was increased in HTN-12wk. Ees was preserved in HTN-5wk and HTN-12wk. τ was increased in HTN-5wk (50±3* ms) and HTN-12wk (62±10* ms) dogs compared with sham (41±2 ms). Plasma Ang II, ET, catecholamines, and plasma renin activity were unchanged during the progressive HTN. Ang II and ET in LV and kidney were not different from controls.

Conclusions—Systemic HTN induces LV hypertrophy, myocardial fibrosis, and isolated diastolic dysfunction in the absence of local or systemic activation of Ang II or ET. These findings suggest that load is the prevailing stimulus for the structural and functional changes associated with early hypertensive heart disease. (Circulation. 2001;104:215-220.)

Key Words: hypertension ■ hypertrophy ■ hemodynamics ■ angiotensin ■ endothelin

Epidemiological studies document that hypertension (HTN) accounts for 39% of heart failure (CHF) cases in men and 59% of cases in women. The effects of systemic HTN on the heart are complex but include left ventricular hypertrophy (LVH), myocardial fibrosis, and impairment in diastolic function. The degree of LVH observed in patients with HTN correlates poorly to blood pressure. This observation has led to speculation that humoral factors modulate the hypertrophic response to pressure overload. Local activation of the renin-angiotensin system (RAS) and endothelin (ET) have been reported in models of pressure overload. Furthermore, angiotensin II (Ang II) and ET are antilusitropic and profibrotic. In contrast, others have asserted that the LVH and diastolic impairment in early hypertensive heart disease are exclusively related to load. Few studies, however, have measured LV load, structure, function, and humoral status to address this controversy.

Our objective was to use a large-animal model of systemic HTN to explore the role of load versus humoral function in early hypertensive heart disease. We hypothesized that systemic HTN would produce LVH and diastolic dysfunction in the absence of local activation of Ang II or ET. This is based on observations in the rapid-pacing model, in which progressive LV remodeling and dysfunction are not associated with local activation of Ang II or ET until end-stage CHF. The present study extends these observations to the study of hypertensive heart disease.

Methods

All experimental procedures were designed in accordance with NIH guidelines and approved by the Mayo Institutional Animal Care and Use Committee.

Model of HTN

The Page renal wrap model of inducing HTN was used. Adult male dogs (20 to 26 kg) were studied (1) 5 weeks after sham surgeries (controls, n=6), (2) 5 weeks after renal wrap (HTN-5wk, n=7), and (3) 12 weeks after renal wrap (HTN-12wk, n=5). Dogs were anesthetized with methohexital sodium 4% solution (12.5 mg/kg) and isoflurane (0.5% to 2.5%) and ventilated (15 mL/kg of 100% Fio2). Via a midline abdominal incision, kidneys were wrapped with silk without constriction of renal vessels. A femoral arterial catheter and port were placed. In controls, the arterial catheter was placed without renal wrap. Torbugesic (0.2 to 0.4 mg/kg every 4 to 6 hours) was administered.
was used for postoperative analgesia. Antibiotics (Cephtabs 500 mg BID×10 days) were given. Blood pressure was measured weekly.

**Echocardiography**

2D targeted M-mode echocardiography (Acuson 128XP/10) was performed in conscious dogs before surgery. Echocardiography was repeated 5 weeks after surgery in controls and HTN-5wk and 5 and 12 weeks after surgery in HTN-12wk dogs. LV end-diastolic (LVDd) and end-systolic (LVDs) internal dimensions, septal wall end-diastolic (Sthd) and end-systolic (Sths) thickness, and posterior wall end-diastolic (PWthd) and end-systolic (PWths) thickness were measured. LV mass was calculated and indexed to body weight: \[ \text{LV mass} = \frac{1}{5.7} \times \left( \text{LVDd}^2 - \text{LVDs}^2 \right) + \left( \frac{1}{2} \times \text{Sthd} - \text{PWthd} - \frac{1}{2} \times \text{Sths} + \frac{1}{2} \times \text{PWths} \right) \]

Humoral Analysis

Before and weekly after surgery, blood was collected in chilled EDTA tubes and centrifuged at 2500 rpm (4°C) for 10 minutes. Radioimmunoassays as previously reported included plasma Ang II, ET-1 (ET), renin activity, and canine brain natriuretic peptide (cBNP). Catecholamines were measured by high-performance liquid chromatography. Tissue homogenates were centrifuged for 30 minutes at 15 000 rpm (4°C), and protein was measured. Supernatants were stored at −20°C.

Blood urea nitrogen (I-STAT, Sensor Devices, Inc) and creatinine (Beckman Creatinine Analyzer) were analyzed.

**Histological Analysis**

Sections were stained with hematoxylin-eosin and Masson’s trichrome. Slides were reviewed by an experienced cardiac pathologist (H.D.T.) blinded to the study group. Fibrosis was graded on a numerical scale: 0 = no fibrosis, 1 = mild fibrosis, 2 = moderate fibrosis, and 3 = significant fibrosis. An experienced renal pathologist (J.P.G.) reviewed the renal histology.

**Statistical Analysis**

Data were averaged and reported as mean±SEM. Comparisons of control and HTN groups were made by Student’s t-tests. Statistical significance was achieved at a value of P<0.05.

**Results**

**Model of Systemic HTN**

The development of HTN is shown in Figure 1. Mean arterial pressure progressively increased in renal-wrapped dogs.

At autopsy, a dense fibrous rind surrounded the renal capsule immediately under the silk. Grossly, the renal capsule and parenchyma were uninvolved in the fibrotic reaction. Histological assessment of renal cortex and medulla revealed no significant interstitial fibrosis, tubular atrophy, or vascular sclerosis in control or hypertensive animals at 5 or 12 weeks. Blood urea nitrogen and creatinine did not change after renal wrapping and were not different from controls at any point (data not shown).

**LV Structure, Function, and Load in the Conscious State**

LV structure, function, and load as assessed at echocardiography in conscious dogs are shown in Table 1. LV mass index increased in HTN-5wk and HTN-12wk dogs without change in LVDd or ejection fraction. Afterload, assessed by end-systolic pressure-volume relationships, end-systolic elastance (Ees), was calculated. The end-diastolic pressure-volume-relationship was fitted monoexponentially, and the chamber stiffness constant, βs, was derived.

LV structure was defined as the top left corner of the pressure-volume loop. The time constant of isovolumic relaxation, τ, was quantified by the method of Glantz as previously described. τ was corrected for heart rate by dividing the value of τ by the square root of the RR interval (in seconds). LV cavity volume (LVV) was calculated:

\[ \text{LVV} = \frac{(\text{LA})}{2} - 3 \times \left( \frac{\text{LV}}{2} \right) \]

Statistical significance was achieved at a value of P<0.05.

**LV Structure: Autopsy and Histology**

The LV mass index at autopsy (Figure 2) was higher in HTN dogs than in controls. The LV log[cBNP], a marker of LVH, was increased in HTN (0.03±0.11 pmol/mg controls versus 1.07±0.32 pmol/mg HTN-5wk, P<0.05, and 0.80±0.26 pmol/mg HTN-12wk, P<0.05). The fibrosis score (Figures 2 and 3) was higher in HTN-12wk than in controls or HTN-5wk.
Systolic and Diastolic Function

Systolic and diastolic function assessed under anesthesia are shown in Table 2. LV peak systolic pressure was increased in HTN. The load-independent index, Ees, was not different between groups. End-artificial elastance was increased in HTN dogs, indicating vasoconstriction. τ was prolonged in HTN-12wk and HTN-12wk dogs. LV end-diastolic pressure (LVEDP) was increased in HTN-12wk. Although LVEDP was higher in HTN-12wk, the coefficient of chamber stiffness (LVEDP) was increased in HTN-12wk. Although LVEDP was significantly different from that in controls (Ees 3 ± 0.2* 54 ± 6 ± 0.6* 250 ± 11† 276 ± 28 mg Hg/mL). Propranolol had a negative lusitropic effect, with increases in τ in all groups (data not shown), but τ was still significantly higher in HTN dogs (58 ± 3 ms HTN-5wk, P.<0.001 versus control; 62±4 ms HTN-12wk, P.<0.01 versus control) than in controls (43±2 ms).

Circulating and Local Humoral Activation

Circulating Ang II, ET (Figure 5), catecholamines, and plasma renin activity in the HTN groups were not increased. Plasma cBNP tended to be slightly higher in HTN dogs, significantly so at 2 weeks (data not shown). Cardiac humoral function is shown in Figure 6. In HTN dogs, Ang II levels were lower in the left atrium and tended to be lower in the LV than in controls. Cardiac ET levels were not different between control and HTN dogs. Renal cortex Ang II (0.48±0.05 pmol/mg controls versus 0.49±0.15 pmol/mg HTN-5wk versus 1.66±0.51 pmol/mg HTN-12wk, P=NS) and ET (1.36±0.26 pmol/mg controls versus 0.95±0.61 pmol/mg HTN-5wk versus 1.92±1.29 pmol/mg HTN-12wk, P=NS) were not significantly activated in HTN dogs. Renal medulla Ang II (0.51±0.08 pmol/mg controls versus 0.63±0.11 pmol/mg HTN-5wk versus 1.78±1.26 pmol/mg HTN-12wk, P=NS) and ET (3.99±0.95 pmol/mg controls versus 3.99±0.72 pmol/mg HTN-5wk versus 1.26±0.52 pmol/mg HTN-12wk, P=NS) were also not activated in HTN dogs.

Discussion

The present study demonstrates that renal wrapping produces sustained HTN without renal parenchymal inflammation or renal insufficiency. Furthermore, the development of HTN is unassociated with systemic or renal activation of the vasoconstrictor hormones Ang II or ET. This model displayed the hallmarks of early hypertensive heart disease: LVH, myocardial fibrosis, and diastolic dysfunction without systolic dysfunction. These changes occurred in the absence of systemic or myocardial activation of prohypertrophic, profibrotic, and antilusitropic neurohormones, Ang II or ET. These data advance our understanding of the effects of HTN on LV structure and function and support the concept that load

TABLE 1. Conscious Assessment of LV Structure, Function, and Load by Echocardiography

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, kg</th>
<th>LVDD, cm</th>
<th>EF, %</th>
<th>LVMI, g/kg body wt</th>
<th>SBP, mm Hg</th>
<th>Ees, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=6)</td>
<td>23±0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.2±0.0</td>
<td>63±3</td>
<td>4.5±0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 5</td>
<td>24.0±6.0</td>
<td>4.1±0.1</td>
<td>58±4</td>
<td>4.6±0.3</td>
<td>174±4</td>
<td>220±16</td>
</tr>
<tr>
<td>HTN-5wk (n=7)</td>
<td>23±0.5</td>
<td>4.0±0.1</td>
<td>58±2</td>
<td>4.2±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24±0.4</td>
<td>3.9±0.1</td>
<td>61±2</td>
<td>6.0±0.4*</td>
<td>231±4†</td>
<td>253±7‡</td>
</tr>
<tr>
<td>HTN-12wk (n=5)</td>
<td>22±0.4</td>
<td>4.2±0.2</td>
<td>57±1</td>
<td>4.9±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22±0.4</td>
<td>4.1±0.2</td>
<td>58±2</td>
<td>7.0±0.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>22±0.6</td>
<td>3.8±0.2*</td>
<td>54±2</td>
<td>6.9±0.6*</td>
<td>250±11†</td>
<td>276±28</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; LVMI, LV mass index; SBP, systolic blood pressure; and Ees, end-systolic wall stress.

*P<0.05 vs baseline; †P<0.05; ‡P=0.07 vs control.
predominates in the genesis of hypertensive heart disease, at least in this early, yet clearly established phase.

Although RAS antagonism is effective in controlling blood pressure and causing regression of LVH in human and experimental HTN, the role of Ang II activation in the pathophysiology of hypertensive heart disease remains controversial. “Subpressor” doses of Ang II were reported to induce hypertrophy in the rat, although alterations in load cannot be excluded in such studies. Studies in aortic-banded rats reported activation of local RAS as evidenced by increases in myocardial ACE mRNA and ACE activity in vitro. Myocardial Ang II, however, was not measured to document that enhanced ACE mRNA resulted in higher levels of active peptide. In the spontaneously hypertensive rat, plasma and myocardial levels of Ang II are low in the early-compensated stage, although LV Ang II does increase late in the natural history of this model, when systolic dysfunction and CHF develop. Although Page HTN was presumed to be a model of RAS activation, the humoral profile of this model has not been well studied. We found no systemic or local activation of the RAS in this model. Furthermore, we found no activation of myocardial Ang II to suggest that the RAS contributes to the LVH, fibrosis, or

**TABLE 2. Invasive Hemodynamic Assessment of LV Function**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=8)</th>
<th>HTN-5wk (n=7)</th>
<th>HTN-12wk (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVPSP, mm Hg</td>
<td>113±4</td>
<td>144±5*</td>
<td>139±6*</td>
</tr>
<tr>
<td>LV+dp/dt, mm Hg/s</td>
<td>1871±114</td>
<td>2168±124</td>
<td>2399±206*</td>
</tr>
<tr>
<td>SV, mL</td>
<td>25±2</td>
<td>22±4</td>
<td>24±5</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>2.4±0.3</td>
<td>2.4±0.4</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>Ees, mm Hg/mL</td>
<td>5±1</td>
<td>7±1</td>
<td>10±4†</td>
</tr>
<tr>
<td>Ea, mm Hg/mL</td>
<td>3±0.3</td>
<td>6±1*</td>
<td>6±1*</td>
</tr>
<tr>
<td><strong>Diastolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>7±0.3</td>
<td>7±1</td>
<td>11±2*</td>
</tr>
<tr>
<td>Tau dp/dt, ms</td>
<td>41±2</td>
<td>50±3*</td>
<td>61±9*</td>
</tr>
<tr>
<td>βc</td>
<td>0.10±0.03</td>
<td>0.11±0.04</td>
<td>0.09±0.02</td>
</tr>
</tbody>
</table>

LVPSP indicates LV peak systolic pressure; SV, stroke volume; CO, cardiac output; Ea, end-arterial elastance; and βc, LV chamber stiffness constant. Values are mean±SEM.

*P<0.05 vs control.
†Not statistically significant; mean contains an outlying value.
Hayashida et al. 28 found variable levels of circulating Ang II in which the hypertrophic response to pressure overload was unabated. 27 These findings complement studies in a 2-kidney, 1-wrap model. Acute Ang II antagonism improved diastolic function, even in dogs with normal plasma Ang II. 15,26 Our findings are also consistent with studies in mice lacking AT1 receptors, in which the hypertrophic response to pressure overload is unabated. 27 These findings complement studies in a model of cardiac unloading in which marked activation of Ang II was activated, myocardial Ang II was not measured. 

It has been suggested that activation of ET may occur in various stages of LV systolic dysfunction, when there is no activation of circulating or local Ang II despite increases in wall stress. 15,26 Our findings are also consistent with studies in mice lacking AT1 receptors, in which the hypertrophic response to pressure overload is unabated. 27 These findings complement studies in a model of cardiac unloading in which marked activation of Ang II was activated, myocardial Ang II was not measured. In contrast, other investigators using bilateral or unilateral renal wrapping have not observed significant fibrosis by use of morphometric techniques 31 and hydroxyproline analysis.14 Our findings suggest that fibrosis starts early in hypertensive heart disease, that it can occur in the absence of profibrotic humoral factors, and that it is associated with hemodynamic abnormalities (increases in LVEDP).

The mechanism responsible for the development of HTN in this model is unclear and deserves comment. When Page and colleagues (Kohlstaedt et al. 32 ) described this model, they demonstrated that the tail artery of a normal dog constricted when perfused with blood from a hypertensive dog, suggesting that a circulating factor was involved. We speculate that the renal manipulation may induce production of an as yet uncharacterized circulating vasoconstrictive factor. This hypothesis is suggested by studies in the pancreas, in which cellophane wrapping induces production of a growth factor that stimulates islet-cell formation and insulin production.33 We are using kidney medulla and cortex extracts to search for possible differential protein expression in the renal-wrap dog. Because of limited canine genomic data, however, further studies in a renal-wrap murine model will be required in identifying differential protein expression. Although at present we are still unable to determine the mechanism of HTN in this model, its hemodynamic features are similar to those of the human condition and thus provide a useful large-animal model of systemic HTN. 

In summary, the absence of local or systemic activation of Ang II or ET in this model suggests that load is the primary determinant of the early ventricular remodeling and diastolic dysfunction associated with systemic HTN. These findings, along with those of the SHEP study, in which control of blood pressure without humoral modulation had a profound effect to reduce progression to CHF,34 suggest aggressive treatment of high blood pressure as the primary means to prevent progression of hypertensive heart disease.

Acknowledgments
This study was supported in part by the NHLBI (1-R01-HL-63281-01A1) and grants from the Mayo Foundation; the Joseph P. and Jeanne M. Sullivan Foundation, Chicago, Ill; the Miami Heart Research Institute; and the National Kidney Foundation of Minnesota, Inc. Dr Hart is a cardiovascular diseases fellow and a recipient of the National Institutes of Health Research Service Award. Dr Redfield is an Established Investigator of the American Heart Association. The authors thank Gail Harty, Denise Heublein, and Sharon Sandberg for their expert technical assistance.
References

Load Versus Humoral Activation in the Genesis of Early Hypertensive Heart Disease
Housmans and Margaret M. Redfield

*Circulation.* 2001;104:215-220
doi: 10.1161/01.CIR.104.2.215

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World
Wide Web at:
http://circ.ahajournals.org/content/104/2/215

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
*Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once
the online version of the published article for which permission is being requested is located, click Request Permissions in
the middle column of the Web page under Services. Further information about this process is available in the Permissions
and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/