

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

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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: $139\pm 4^*$ versus 123 ± 4 mm Hg), week 5 ($174\pm 7^*$ versus 124 ± 4 mm Hg), and week 12 ($181\pm 12^*$ 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 ($50\pm 3^*$ ms) and HTN-12wk ($62\pm 10^*$ 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.¹ The effects of systemic HTN on the heart are complex² 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.² This observation has led to speculation that humoral factors modulate the hypertrophic response to pressure overload. Local activation of the renin-angiotensin system (RAS)³⁻⁵ and endothelin (ET)⁶ have been reported in models of pressure overload. Furthermore, angiotensin II (Ang II)^{7,8} and ET^{9,10} are anti-lisotropic and profibrotic.^{11,12} In contrast, others have asserted that the LVH¹³ and diastolic impairment in early hypertensive heart disease are exclusively related to load.¹⁴ 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.^{15,16} 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 Page¹⁷ 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)

Received November 30, 2000; revision received March 14, 2001; accepted March 28, 2001.

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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 (Sth_d) and end-systolic (Sth_s) thickness, and posterior wall end-diastolic ($PWth_d$) and end-systolic ($PWth_s$) thickness were measured. LV mass was calculated and indexed to body weight¹⁸: $LV\ mass = [(LVDd + Sth_d + PWth_d)^3 - (LVDd)^3] \times 1.04 \times 0.80$. Ejection fraction was calculated¹⁹ as $EF = 100 \times (LVDd^2 - LVDs^2) / LVDd^2$. End-systolic wall stress was estimated by a cylindrical model²⁰: $Es\sigma\ (g/cm^2) = 1.36 \times [(SBP \times LVDs) / (2 \times hes)]$, where SBP is systolic blood pressure measured at echocardiography and $hes = (Sth_s + PWth_s) / 2$.

Acute Experimental Protocol

Fasted dogs were anesthetized with fentanyl 0.25 mg/kg (Johnson Matthey, Inc) and midazolam 0.75 mg/kg IV (Roche) and ventilated (Harvard Respirator) with room air and O₂. Infusion of fentanyl 0.18 mg · kg⁻¹ · h⁻¹ and midazolam 0.59 mg · kg⁻¹ · h⁻¹ was titrated to effect. Via a left thoracotomy, an LV pressure transducer (Konigsberg Instruments, Inc) was inserted in the apex. A fluid-filled pigtail catheter (USCI) was used to calibrate the transducer. Piezoelectric crystals (2.0 mm) (Sonometrics Corp) were implanted on anterior and posterior endocardial surfaces at the mid-LV level, the endocardial LV apex, and basal epicardial surfaces. An occluder was placed around the inferior vena cava. Dogs were atrial-paced at 20 bpm above sinus rate.

Steady-state data were collected over 5 minutes. A 250-mL IV bolus of warmed saline was given. Three inferior vena cava occlusions producing reduction of peak LV systolic pressure of ≥ 30 mm Hg were obtained. Steady-state and variable preloaded measurements were repeated after administration of propranolol (2 mg/kg IV).

Dogs were killed by removal of the heart under anesthesia, consistent with guidelines of the Panel on Euthanasia of the American Veterinary Medical Association. The LV mass index was calculated (LV mass/body wt, g/kg). Renal and myocardial samples were placed in formalin or frozen in liquid nitrogen and stored at -80°C .

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^{15,21} 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.¹⁵ 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.

Hemodynamic Analysis

Data acquisition systems included CA Recorder (Data Integrated Scientific Systems) and SonoLab (Sonometrics Corp). Signals were digitized at 250 Hz and analyzed by Spectrum 1.0 (Wake Forest University) or CardioSoft (Sonometrics Corp). End diastole was

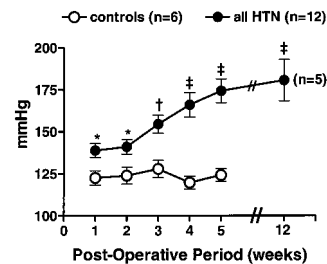


Figure 1. Mean arterial pressure after sham surgery (control) or renal wrapping (HTN). Values are mean±SEM. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ vs controls.

defined as relative minimum of LV pressure after the A wave. End systole 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.²² 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: $LVV = (\pi/6)(LA)(SA)^2$, where LA is the long axis and SA is the anteroposterior short axis. The slope of end-systolic pressure-volume relationships, end-systolic elastance (E_{es}), was calculated.²² The end-diastolic pressure-volume relationship was fitted monoexponentially, and the chamber stiffness constant, β_c , was derived.

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 wall stress ($Es\sigma$) tended to be increased in both HTN groups.

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.

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

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

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

* $P < 0.05$ vs baseline; † $P < 0.05$, ‡ $P = 0.07$ vs control.

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-arterial elastance was increased in HTN dogs, indicating vasoconstriction. τ was prolonged in HTN-5wk 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 was not different between HTN groups and controls.

Systolic and diastolic function were reassessed after β -adrenergic blockade with propranolol (Figure 4). When differences in sympathetic tone were controlled for by propranolol, inotropic function in HTN dogs (Ees 5±1 mm Hg/mL HTN-5wk, 8±3 mm Hg/mL HTN-12wk) was not significantly different from that in controls (Ees 3±0.3 mm 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, $P = \text{NS}$) 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, $P = \text{NS}$) 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, $P = \text{NS}$) 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, $P = \text{NS}$) were also not activated in HTN dogs.

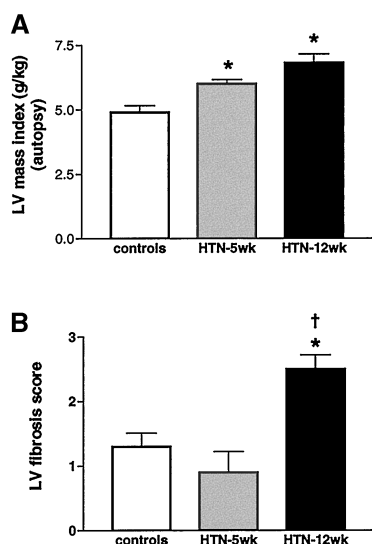


Figure 2. LV mass index (g/kg) (A) in controls, HTN-5wk, and HTN-12wk. * $P < 0.05$ vs controls. LV fibrosis score (B) in controls, HTN-5wk, and HTN-12wk. * $P < 0.01$ vs controls; † $P < 0.01$ vs HTN-5wk.

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 antilutotropic 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

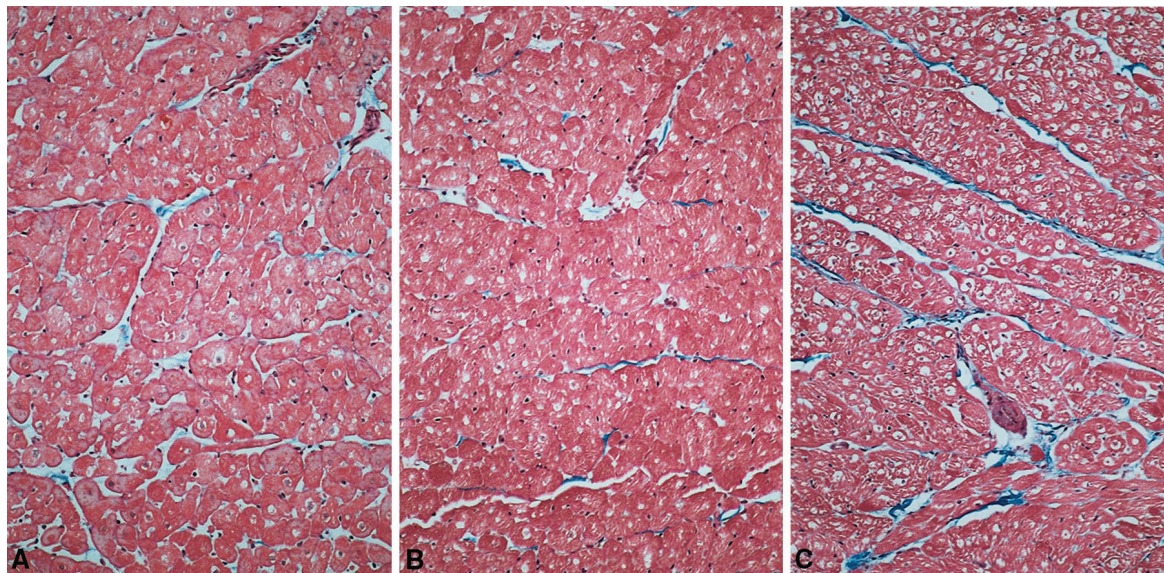


Figure 3. Photomicrographs of LV myocardium. Control animals have little interstitial connective tissue (A). HTN-5wk group has minimal fibrosis (B); HTN-12wk dogs have moderate fibrosis (C). Masson's trichrome stain: collagen stains deep blue. Magnification $\times 20$.

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,²³ 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.²⁴ Studies in aortic-banded rats reported activation of local RAS as evidenced by increases in myocardial ACE mRNA and ACE activity in vitro.^{4,5} 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.²⁵ Although Page HTN was presumed to be a model of RAS activation,^{14,17} 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

	Controls (n=8)	HTN-5wk (n=7)	HTN-12wk (n=5)
Systolic function			
LVPS, mm Hg	113 \pm 4	144 \pm 5*	139 \pm 6*
LV+dP/dt, mm Hg/s	1871 \pm 114	2168 \pm 124	2399 \pm 206*
SV, mL	25 \pm 2	22 \pm 4	24 \pm 5
CO, L/min	2.4 \pm 0.3	2.4 \pm 0.4	2.3 \pm 0.3
Ees, mm Hg/mL	5 \pm 1	7 \pm 1	10 \pm 4†
Ea, mm Hg/mL	3 \pm 0.3	6 \pm 1*	6 \pm 1*
Diastolic function			
LVEDP, mm Hg	7 \pm 0.3	7 \pm 1	11 \pm 2*
Tau dP/dt, ms	41 \pm 2	50 \pm 3*	61 \pm 9*
β_c	0.10 \pm 0.03	0.11 \pm 0.04	0.09 \pm 0.02

LVPS indicates LV peak systolic pressure; SV, stroke volume; CO, cardiac output; Ea, end-arterial elastance; and β_c , LV chamber stiffness constant. Values are mean \pm SEM.

* $P < 0.05$ vs control.

†Not statistically significant; mean contains an outlying value.

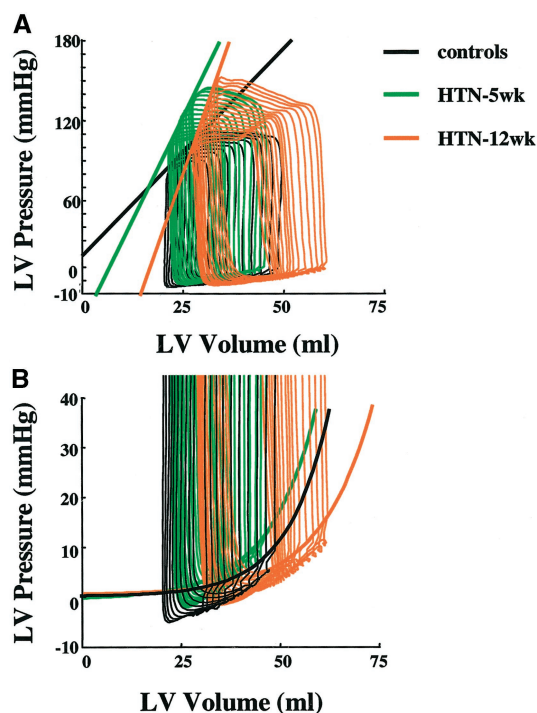


Figure 4. Representative end-systolic (A) and end-diastolic (B) pressure-volume relationships of control, HTN-5wk, and HTN-12wk dogs in presence of β -adrenergic receptor blockade.

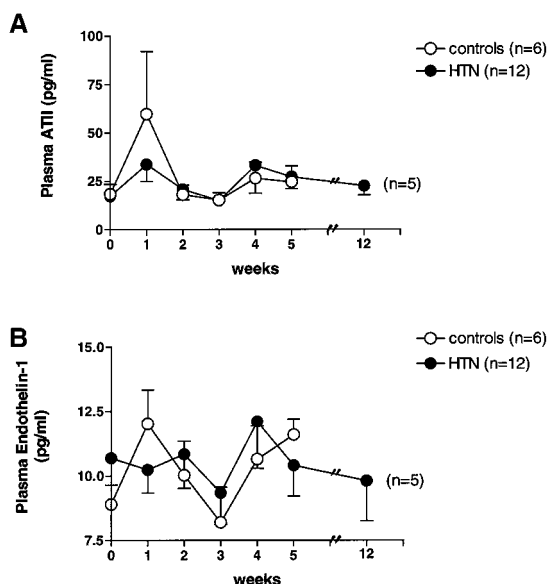


Figure 5. Circulating humoral response. Ang II (A) and ET-1 (B) concentrations measured weekly after surgeries until week 5 in controls and HTN-5wk and again at 12 weeks in HTN-12wk (n=5).

diastolic dysfunction evident in early hypertensive heart disease.

These findings are reminiscent of those observed in early 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 and ET did not prevent cardiac atrophy.²¹ In contrast, Hayashida et al²⁸ found variable levels of circulating Ang II in the 2-kidney, 1-wrap model. Acute Ang II antagonism improved diastolic function, even in dogs with normal plasma Ang II. Although these investigators speculated that the cardiac RAS was activated, myocardial Ang II was not measured.

It has been suggested that activation of ET may occur in this form of HTN.¹⁰ Although plasma ET levels were not increased, it was proposed that local ET activation might occur.¹⁰ Although we did not assess all vascular beds, we found no evidence of ET activation in myocardial or renal tissue. These findings do not support the participation of ET in this form of hypertensive heart disease.

Both LVH and increased fibrosis have been suggested to increase LV stiffness²⁹ in hypertensive heart disease. In our study, fibrosis was increased in the HTN-12wk group, in which the LVH at autopsy was slightly (though not significantly) greater. Although we were unable to demonstrate an

increase in the LV stiffness constant, LVEDP was higher, suggesting early decreases in LV compliance or altered distensibility. This finding is consistent with those of Abrahams et al,³⁰ who found altered LV collagen matrix in monkeys as early as 4 weeks after unilateral renal wrap. In contrast, other investigators using bilateral or unilateral renal wrapping have not observed significant fibrosis by use of morphometric techniques³¹ 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 al³²) 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.

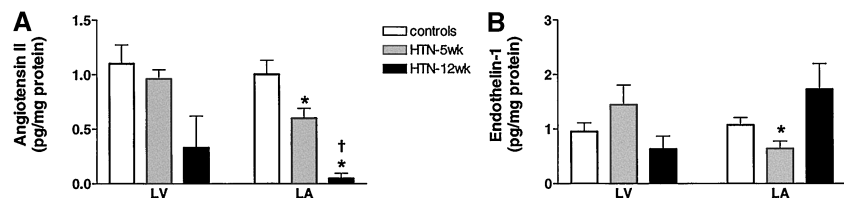


Figure 6. Cardiac humoral response. Tissue concentrations of Ang II (A) and ET-1 (B) are normalized to tissue protein content for controls, HTN-5wk, and HTN-12wk groups. Values are mean±SEM. LA indicates left atrium. *P<0.05 vs controls, †P<0.05 vs HTN-5wk.

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