Inotropic Response to Norepinephrine Is Augmented Early and Maintained Late in Conscious Dogs With Perinephritic Hypertension

Richard P. Shannon, Ricardo J. Gelpi, Luc Hittinger, Dorothy E. Vatner, Charles J. Homcy, Robert M. Graham, and Stephen F. Vatner

We studied the inotropic responses to intravenous infusions of norepinephrine in nine conscious chronically instrumented dogs before and early (2–4 weeks) in the development of perinephritic hypertension; seven conscious dogs were studied later (~14 weeks), during a more stable phase of hypertension. Perinephritic hypertension was associated with a 24% increase in left ventricular (LV) mass during developing hypertension; no further increase was seen during the stable hypertension phase. LV end-systolic stress was increased early (p<0.01) but was normalized later. The LV end-systolic stress–volume relation demonstrated an enhanced contractile response to norepinephrine during developing hypertension, which returned toward control later in the course of stable hypertension. The LV dP/dt responses to norepinephrine (0.4 µg/kg/min) were significantly greater during developing hypertension (7,509±337 mm Hg/sec, p<0.05) compared with the control period (4,737±286 mm Hg/sec) and returned toward the control value during stable hypertension (5,168±465 mm Hg/sec). The enhanced inotropic responses to norepinephrine in developing hypertension were preserved in the presence of ganglionic blockade, suggesting that the augmentation was not mediated via reflex mechanisms. These physiological responses were associated with an increase in β-adrenergic receptor density, but no significant change in basal or maximal adenylate cyclase stimulation occurred during developing hypertension. Thus, in contrast to prior studies in anesthetized animals, the inotropic response to β-adrenergic stimulation is not depressed in conscious dogs but is enhanced selectively during the development of hypertension and maintained during stable hypertension. (Circulation Research 1991;68:543–554)

Myocardial hypertrophy is a complex pathophysiological response to excessive cardiac load, designed to normalize ventricular wall stress and maintain normal systolic function. However, the hypertrophic process is heterogeneous in terms of its morphological, biochemical, and functional consequences and differs in systemic hypertension from other causes of cardiac hypertrophy.1,2 Recent evidence from our laboratory3 revealed that left ventricular (LV) systolic function is enhanced at baseline early (2–4 weeks) in the development of cardiac hypertrophy, secondary to perinephritic hypertension, in conscious dogs and that the enhanced inotropic state at baseline was independent of altered loading conditions but was dependent on the integrity of the sympathetic nervous system. Either ganglionic or β-adrenergic blockade abolished the augmented response at baseline.3 These data raise the question as to whether adrenergic responsiveness is enhanced during the development of cardiac hypertrophy secondary to systemic hypertension and whether adrenergic responsiveness is maintained or altered later in the course of hypertension when compensated LV hypertrophy is established. Prior studies using genetic or renovascular models of hypertension in the rat have suggested a reduced myocardial response to adrenergic stimulation4–11 associated with decreases in β-receptor density and/or affinity.6,10–15 However, these studies are con-
founded by methodologies using either isolated hearts or papillary muscle preparations or are mitigated by the use of anesthesia in intact animal preparations. In addition, there are important species differences: the rat exhibits more prominent α-adrenergic receptor control of LV contractility,7,15,16,17 as well as alterations in myocardial isoenzyme forms that occur during hypertrophy.18

Thus, we first sought to establish whether β-adrenergic responsiveness to the endogenous sympathetic neurotransmitter, norepinephrine, is altered both early, during the development of perinephritic hypertension, and later, during stable hypertension. A second aim was to determine if the alterations in response to norepinephrine could be attributed to alterations in autonomic reflex buffering. This was addressed by reexamining responses to norepinephrine in the presence and absence of ganglionic blockade. Finally, the physiological responses were correlated with changes in myocardial β-adrenergic receptor density.

Materials and Methods

Instrumentation

Twenty-four mongrel dogs of either sex, weighing between 20 and 31 kg, were sedated with xylazine (2 mg/kg i.m.) and anesthetized using halothane anesthesia (1 vol%). With the use of sterile technique and through an incision in the left fifth intercostal space, Tygon catheters (Norton Plastics and Synthetic Division, Akron, Ohio) were implanted in the descending thoracic aorta and left atrium. In all dogs, piezoelectric ultrasonic dimension crystals were implanted on opposing anterior and posterior endocardial surfaces of the LV to measure the internal short axis and on opposing endocardial and epicardial surfaces in the same equatorial plane as the internal short axis diameter crystals to measure wall thickness. The endocardial wall-thickness crystals were implanted obliquely to avoid damage to the myocardium between the two wall-thickness crystals. Ultrasonic transducers were also implanted at the basal epicardial surface and apical endocardial surface to measure LV long axis. A solid-state miniature pressure transducer (model P22. Konigsberg Instruments, Inc., Pasadena, Calif.) was implanted in the apex to measure LV pressure in all dogs (Figure 1). The thoracotomy was closed, and the dogs were allowed to recover for 2–3 weeks. The dogs used in this study were maintained in accordance with the guidelines, Guide for the Care and Use of Laboratory Animals, of the Institute of Laboratory Animal Resources, National Council (Department of Health and Human Services publication No. [NIH] 85-23, revised 1985), and the Standing Committee on Animal Care of Harvard Medical School.

Aortic and left atrial pressures were sampled from chronically implanted catheters and measured with strain-gauge manometers (model P23 1D, Statham Instruments, Oxnard, Calif.), which were calibrated with a mercury manometer. LV pressure was measured using the solid-state miniature pressure gauge calibrated in vitro with a mercury manometer and in vivo using the left atrial and aortic catheters and Statham strain-gauge manometers.19,20 An ultrasonic dimension gauge was used to measure LV dimensions.21 The dimension gauge generates a voltage linearly proportional to the transit time of ultrasonic impulses traveling at the velocity of 1.58×106 m/sec between the crystals. At constant room temperature, the thermal drift of the instrument is minimal, that is, less than 0.02 mm in 6 hours. Any drift in the measurement system was eliminated during the experiment by periodic calibration accomplished by substituting impulses of known duration from a crystal-controlled pulse generator having a stability of 0.001%. The position of all transducers was confirmed at autopsy.

Model of Perinephritic Hypertension

After completion of control studies in the normotensive state, perinephritic hypertension was induced in nine mongrel dogs according to the method introduced by Page22 and used previously in our laboratory.3 After removal of perinephric fat, the left kidney was wrapped loosely in a silk pouche through a left flank incision. Care was taken to avoid inadvertent stenosis of the renal artery. One week later, a contralateral nephrectomy was performed through a right flank incision. Technical limitations and long-term morbidity precluded the study of these same dogs during a more chronic phase of hypertension. Accordingly, a separate group of seven dogs was made hypertensive initially by performing a simultaneous bilateral renal wrap, as described above, through a midline laparotomy. Subsequently, these dogs underwent chronic instrumentation at 10–14 weeks after developing hypertension, thereby avoiding the morbidity associated with chronic instrumentation and renal insufficiency over the prolonged period of hypertension. All surgical procedures were performed using sterile surgical techniques, and general anesthesia was induced by halothane (1 vol%). An additional five dogs were prepared as sham hypertensive controls; that is, the left kidney was dissected but not wrapped, and the right kidney was removed 1 week later. An additional three dogs were instrumented fully for a period of 8 weeks and were included as sham-operated controls. Together, these eight dogs were used as a separate comparison group for the stable hypertensive dogs and for pathological and biochemical measurements.

Protocols

Nine dogs were studied as controls in a normotensive state beginning 2–3 weeks after instrumentation, when they were healthy and had completely recovered from the effects of surgery. The same nine dogs were studied 2–4 weeks after induction of perinephritic hypertension (renal wrap and contralateral nephrectomy). An additional seven dogs were studied during stable hypertension, that is, approximately
FIGURE 1. Schematic illustration showing instrumentation and representative tracings from one dog. LV, left ventricular.
14 weeks after induction of hypertension. At no time were significant electrolyte disturbances or renal insufficiency noted.

During the control period and during both developing and stable hypertension, each dog received a graded infusion of norepinephrine (0.05, 0.1, 0.2, and 0.4 μg/kg/min). On a separate occasion, the responses to norepinephrine were determined in the presence of ganglionic blockade effected by hexamethonium (20 mg/kg) and atropine methylbromide (0.1 mg/kg). The efficacy of ganglionic blockade was established by demonstrating the absence of a heart rate response to the hypotensive effects of bolus administration of nitroglycerin (120 μg). Finally, on a separate occasion, each dog received a graded intravenous infusion of phenylephrine (1, 2, and 5 μg/kg/min) to study the hemodynamic and inotropic responses to α-adrenergic stimulation. These studies were designed to investigate the potential role that α-adrenergic stimulation might play in the observed responses to norepinephrine. Similar protocols were used in the eight sham-operated dogs, which were used as comparisons for the responses in the stable hypertensive dogs.

Biochemical Preparation

The left ventricle and septum were weighed, trimmed of fat and connective tissue, minced, and homogenized in 4 vol buffer I (0.75 M NaCl and 10 mM histidine, pH 7.5) with a Polytron (model PT-20S, Brinkmann Instruments, Inc., Westbury, N.Y.) for 5 seconds at half speed. The homogenate was centrifuged at 14,000g for 20 minutes. The pellet was resuspended in buffer I, homogenized for 5 seconds at half speed, and centrifuged at 14,000g for 20 minutes. The pellet was homogenized and centrifuged as before. The pellet was resuspended in buffer II (10 mM NaHCO₃ and 5 mM histidine), homogenized for 30 seconds three times at half speed, and centrifuged at 14,000g for 20 minutes. The pellet was filtered through one layer of Japanese silk screen, size 12, and saved as the crude membranes.

All studies were performed in triplicate in the presence of Tris buffer (100 mM Tris, 1 mM EGTA, and 5 mM MgCl₂, pH 7.2). β-Adrenergic receptor antagonist binding studies were performed using eight concentrations of 25 μl of [125I]cyanopindolol ranging from 0.02 to 1.0 nM, 25 μM isoproterenol (0.1 mM) or buffer, and 100 μl crude membrane protein (10 μg/assay). The antagonist binding data were analyzed by the LIGAND program. A linear regression was performed on the amount bound versus bound/free ligand. An r² value of 0.7 was the criterion used for acceptability of the data.

Adenylate cyclase activity was assayed according to the method of Salomon et al. Dose–response curves were performed for each stimulator of adenylate cyclase activity to determine that maximally stimulated activity would be measured. The concentrations of the following compounds were found to produce maximally stimulated adenylate cyclase activity (mM): GTP 0.1, GTP 0.1 plus isoproterenol 0.1, Gpp(NH)p 0.1, NaF 10, and forskolin 0.1.

Tissue and plasma catecholamines were measured by the radioenzymatic assay of Peuler and Johnson. The data were recorded on a multichannel tape recorder (model 101, Honeywell, Denver) and on a direct-writing oscillograph (Mark 200, Gould-Brush, Cleveland, Ohio). A cardiogammeter (model 9857B, Beckman Instruments, Inc., Fullerton, Calif.), triggered by the LV pressure pulse, provided a continuous recording of heart rate. Continuous recordings of LV dp/dt and LV dV/dt were derived from LV pressure signals and from the short axis dimension signals, respectively, using Philbrick operational amplifiers (Teledyne Philbrick, Dedham, Mass.), which were operated as differentiators and had a frequency response of 700 Hz. A triangular wave signal was substituted for the pressure and dimension signals to calibrate the differentiator directly. LV end-diastolic dimensions were measured immediately before the onset of ventricular contraction. LV end-systolic dimensions were measured at the time of maximum negative dp/dt. Ejection time was taken as the interval between maximum and minimum LV dp/dt.

Cavity volume was calculated using an ellipsoidal model:

\[
EDV = \frac{(\pi/6)(EDD)^2(EDL - 0.55 \times EDW)}{1000}
\]

\[
ESV = \frac{(\pi/6)(ESD)^2(ESL - 0.55 \times ESW)}{1000}
\]

where EDV is end-diastolic volume, ESV is end-systolic volume, EDD is the end-diastolic short axis, ESL is the end-systolic short axis, EDL is the end-diastolic long axis, ESL is the end-systolic long axis, EDW is end-diastolic wall thickness, and ESW is end-systolic wall thickness. Notice that a wall thickness factor is subtracted from the measured long axis because one of the long axis crystals is endocardial and the second is epicardial.

End-systolic wall stress (Es σ) was calculated by using a cylindrical model:

\[
Esσ = 1.36 \times \left[ \frac{(AP_{es} \times SA_{es})}{(2 \times WTH_{es})} \right]
\]

where AP es is the aortic pressure at end systole, SA es is the short axis diameter at end systole, and WTH es is wall thickness at end systole.

The individual end-systolic stress–volume relations for each dog were generated from playback of on-line acquired data during both phenylephrine and norepinephrine infusions. End systole was defined as the maximum stress–volume point. The end-systolic stress–volume points were averaged, and the relation
TABLE 1. Pathology

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=8)</th>
<th>Developing HTN (n=9)</th>
<th>Stable HTN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (kg)</td>
<td>26±1</td>
<td>25±1</td>
<td>23±1</td>
</tr>
<tr>
<td>LV free wall wt (g)</td>
<td>92±7</td>
<td>114±6*</td>
<td>111±3*</td>
</tr>
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<td>LV wt/body wt (g/kg)</td>
<td>3.6±0.2</td>
<td>4.5±0.2*</td>
<td>4.7±0.2*</td>
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<tr>
<td>LV+septum/body wt (g/kg)</td>
<td>4.8±0.3</td>
<td>6.1±0.2*</td>
<td>5.9±0.2*</td>
</tr>
<tr>
<td>RV wt (g)</td>
<td>45±3</td>
<td>48±3</td>
<td>45±3</td>
</tr>
<tr>
<td>RV wt/body wt (g/kg)</td>
<td>1.8±0.1</td>
<td>1.9±0.2</td>
<td>1.9±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Sham, eight sham-operated control dogs; developing HTN, nine dogs studied early (2–4 weeks) in the development of perinephritic hypertension; stable HTN, dogs studied later (~14 weeks) in the development of perinephritic hypertension; LV, left ventricular; RV, right ventricular.

*p<0.05 compared with sham-operated control values.

was established for each group at control and during developing and stable hypertension. The slope of the individual relations was determined as the end-systolic elastance (Ees).29,30

Statistical Analyses

All analyses31 were performed using BMDP biomedical computer programs (BMDP, Los Angeles). The group of sham dogs and the group of dogs studied at control were compared with Hotelling’s T² test. This test showed no significant difference between these two groups at p<0.05. The comparisons performed to study the differences between effects in the different groups were as follows: first, the comparison of the paired data (normotensive control period versus period of developing hypertension); then, the comparison between the stable hypertension group and the sham group (used as the normotensive control for the stable hypertension group); and finally, the comparison between developing and stable hypertension groups. Significance was set at p<0.05. The method of statistical analysis was the repeated-measures analysis of variance. This was followed, when required, by an analysis of linear contrasts to compare the baseline with the drug responses and, when required, by Student’s t test to compare two groups at each drug response level. The individual slopes (Ees) of the end-systolic stress-volume relation were compared in the same dogs at control and during developing hypertension using a paired Student’s t test corrected, when necessary, for multiple comparisons.

Results

Pathology

Table 1 reveals the postmortem heart weights in each of the three study groups. There were no differences in body weight, but there was a 24% increase in LV free wall mass in developing hypertension (p<0.05) and a similar significant increase in stable hypertension (p<0.05). Both the LV free wall and free wall plus septum to body weight ratios were significantly greater in the two hypertensive groups compared with sham-operated controls (p<0.05). There were no differences in the right ventricular weights nor the right ventricular weight/body weight ratios among the three groups.

Baseline Hemodynamics

Table 2 lists the hemodynamics and LV contractile indexes at baseline in the groups. Baseline LV peak systolic, end-diastolic, and mean arterial pressures were significantly greater (p<0.01) in dogs that were

TABLE 2. Effects of Hypertension on Baseline Hemodynamics and Left Ventricular Function

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>Developing HTN (n=9)</th>
<th>Sham HTN (n=8)</th>
<th>Stable HTN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>124±3</td>
<td>160±5*</td>
<td>121±3</td>
<td>161±6†</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>8±1</td>
<td>17±2*</td>
<td>8±1</td>
<td>11±2</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>96±3</td>
<td>128±3*</td>
<td>97±3</td>
<td>131±7*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>94±3</td>
<td>83±5</td>
<td>96±5</td>
<td>105±2</td>
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<tr>
<td>LV end-diastolic diameter (mm)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Short axis</td>
<td>40.0±1.4</td>
<td>42.8±1.2</td>
<td>39.8±0.7</td>
<td>39.8±1.5</td>
</tr>
<tr>
<td>Long axis</td>
<td>63.7±2.7</td>
<td>65.4±3.1</td>
<td>65.1±3.1</td>
<td>67.1±2.0</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short axis</td>
<td>29.8±1.4</td>
<td>30.8±1.4</td>
<td>30.4±0.6</td>
<td>29.4±1.7</td>
</tr>
<tr>
<td>Long axis</td>
<td>60.6±2.7</td>
<td>62.7±2.8</td>
<td>62.3±2.9</td>
<td>64.4±1.9</td>
</tr>
<tr>
<td>LV end-diastolic wall thickness (mm)</td>
<td>12.3±0.5</td>
<td>12.5±0.4</td>
<td>12.2±0.9</td>
<td>13.7±0.5</td>
</tr>
<tr>
<td>LV end-systolic wall thickness (mm)</td>
<td>14.2±0.7</td>
<td>15.5±0.8</td>
<td>14.9±1.0</td>
<td>17.4±0.4</td>
</tr>
<tr>
<td>LV end-systolic stress (g/cm²)</td>
<td>152±11</td>
<td>203±18*</td>
<td>158±9</td>
<td>162±17</td>
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<tr>
<td>LV +dP/dt (mm Hg/sec)</td>
<td>3,011±112</td>
<td>4,068±213*</td>
<td>3,187±92</td>
<td>3,202±238</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Control, dogs studied before induction of perinephritic hypertension; developing HTN, dogs from control group studied early (2–4 weeks) in the development of perinephritic hypertension; sham, separate group of sham-operated control dogs; stable HTN, separate group of dogs studied later (~14 weeks) in the development of perinephritic hypertension.

*p<0.01 developing hypertension compared with control values.

†p<0.01 stable hypertension compared with sham values.
studied during both developing and stable perinephritic hypertension. There were no differences in heart rate among the groups. During developing hypertension, there was a significant increase in LV end-systolic wall stress compared with the control value (p<0.01). End-systolic wall stress was normalized later during the stable phase of perinephritic hypertension, as a consequence of changes in LV geometry, despite similar increases in systolic pressure. During developing hypertension, there were increases of a similar magnitude in both wall thickness and LV cavity diameter, such that the ratio of wall thickness to cavity diameter was not different compared with the control value. During stable hypertension, LV cavity diameter returned to control levels, whereas there were further increases in wall thickness, both at end diastole and end systole, such that the ratio of cavity diameter to wall thickness declined significantly (p<0.05). Thus, during stable hypertension, despite the persistently elevated LV pressures, end-systolic wall stress was normalized to control levels through a proportionally greater increase in LV wall thickness compared with LV short axis diameter. There were no significant alterations in long axis diameter.

At baseline, the isovolumic index of contractility, LV dP/dt, was significantly greater (p<0.01) during developing hypertension compared with the control period. Furthermore, there was no depression in baseline LV dP/dt between sham-operated dogs and dogs studied late in the course of stable hypertension.

Response to Norepinephrine

Because important alterations in loading conditions occurred during the course of hypertension, the end-systolic stress–volume relations, a relatively load-independent index of LV contractility,29,30 were constructed in response to norepinephrine. Figure 2 depicts the average relation for control dogs in the normotensive state and for the same dogs studied during developing perinephritic hypertension. The relation is also depicted for the dogs with stable hypertension compared with controls. At baseline, the relations were established based on changes in stress–volume loops constructed during an infusion of phenylephrine. At baseline, there was an increase in Ees during the period of developing hypertension (27.8±0.5 g/cm²/ml) compared with the control period (16.9±2.5 g/cm²/ml), although the difference was not significant. These data suggest an enhanced contractile state at baseline in developing hypertension compared with the control period, although of a lesser magnitude than noted in our prior findings.3 Later, in the course of stable hypertension, the end-systolic stress–volume relations (Ees=17.2±1.6 g/cm²/ml) returned toward the control value. In contrast, in the course of developing hypertension, Ees was further increased (44.6±10.6 g/cm²/ml) in response to the positive inotropic effect of norepinephrine compared with the response in normotensive control studies (17.9±5.1 g/cm²/ml; p<0.05). The relation returned to control levels during the later, more stable phase of hypertension (26.3±3.2 g/cm²/ml). Thus, the developing course of perinephritic hypertension is associated with a shift in the average end-systolic stress–volume relation in response to norepinephrine, reflecting an enhanced inotropic responsiveness to adrenergic stimulation. The inotropic state is maintained at or above control levels later in hypertension. In either case, there is no evidence of depressed LV systolic function over the nearly 4-month period of perinephritic hypertension, when using an index that is relatively independent of load.

Table 3 depicts the hemodynamic responses to increasing doses of intravenous norepinephrine.
There were dose-related increases in LV systolic, end-diastolic, and mean arterial pressures and LV end-diastolic wall stress in each group. Of note is the finding that the magnitude of the pressor response to norepinephrine was significantly greater ($p < 0.05$) in studies during developing hypertension compared with control studies. The increases in LV dP/dt in response to intravenous norepinephrine were significantly greater during developing hypertension compared with the normotensive control period, whereas responses to intravenous norepinephrine were maintained later during the stable hypertensive phase, suggesting again that there was no depression in adrenergic responsiveness over the period studied (Table 3). Figure 3 depicts the response of the isovolumic index, LV dP/dt, to intravenous norepinephrine, expressed as the change from baseline. There remained a significantly enhanced response during developing hypertension compared with the control period, with preservation of adrenergic responsiveness to norepinephrine seen later in hypertension. Similar significant differences were noted when the data were expressed as percent change from baseline, suggesting that the enhanced responsiveness to norepinephrine seen in developing hypertension was not attributable to the augmented contractile state noted at baseline.

To establish whether the enhanced responsiveness to norepinephrine during developing hypertension was due to direct myocardial effects or mediated via autonomic reflex mechanisms, the dose–response relation to norepinephrine was examined under ganglionic blockade produced by hexamethonium and atropine methylbromide. Expressed as a change from baseline (Figure 4), the LV dP/dt response to norepinephrine (0.4 μg/kg/min) in developing hypertension

### Table 3. Hemodynamic Responses to Norepinephrine Infusions Before and After the Development of Perinephritic Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>0.05</th>
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<td><strong>LV systolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124±3</td>
<td>134±3*</td>
<td>142±4*</td>
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<td>Developing HTN</td>
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<td>170±5*</td>
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<tr>
<td><strong>LV end-diastolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>9±1*</td>
<td>11±1*</td>
<td>14±2*</td>
<td>18±2*</td>
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<td>Developing HTN</td>
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<tr>
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<td><strong>Mean arterial pressure (mm Hg)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Control</td>
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<td>102±3*</td>
<td>109±3*</td>
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<td>205±9‡</td>
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<td>113±3*</td>
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<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
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<td>96±6</td>
<td>92±8</td>
<td>91±7</td>
</tr>
<tr>
<td>Sham HTN</td>
<td>96±5</td>
<td>92±6</td>
<td>82±3*</td>
<td>89±4</td>
<td>86±6</td>
</tr>
<tr>
<td><strong>LV end-systolic stress (g/cm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>152±11</td>
<td>171±10*</td>
<td>188±10*</td>
<td>216±10*</td>
<td>236±14*</td>
</tr>
<tr>
<td>Developing HTN</td>
<td>203±18‡</td>
<td>235±19‡</td>
<td>265±22‡</td>
<td>295±21‡</td>
<td>320±21‡</td>
</tr>
<tr>
<td>Stable HTN</td>
<td>162±17</td>
<td>178±14*</td>
<td>209±19*</td>
<td>268±28*</td>
<td>284±24*</td>
</tr>
<tr>
<td>Sham HTN</td>
<td>158±9</td>
<td>171±8*</td>
<td>192±9*</td>
<td>239±18*</td>
<td>244±17*</td>
</tr>
<tr>
<td><strong>LV dP/dt (mm Hg/sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3,011±112</td>
<td>3,150±115*</td>
<td>3,255±105*</td>
<td>3,831±177*</td>
<td>4,737±286*</td>
</tr>
<tr>
<td>Developing HTN</td>
<td>4,068±213†</td>
<td>4,312±222†</td>
<td>4,738±229†</td>
<td>5,752±326†</td>
<td>7,509±337†</td>
</tr>
<tr>
<td>Stable HTN</td>
<td>3,202±238</td>
<td>3,250±229</td>
<td>3,459±248*</td>
<td>4,283±267*</td>
<td>5,168±465*</td>
</tr>
<tr>
<td>Sham HTN</td>
<td>3,187±92</td>
<td>3,220±115</td>
<td>3,381±103*</td>
<td>4,134±147*</td>
<td>4,953±212*</td>
</tr>
</tbody>
</table>
remained enhanced (+7,423±777 mm Hg/sec) under ganglionic blockade compared with the normotensive control period (+4,938±662 mm Hg/sec) and was maintained later in the course of perinephritic hypertension (+4,937±290 mm Hg/sec). Thus, the enhanced responsiveness to norepinephrine that was seen during developing hypertension was not abolished by ganglionic blockade, suggesting that the augmentation was due in part to direct myocardial effects of norepinephrine. Interestingly, in the presence of ganglionic blockade, heart rate increases were greater during developing hypertension (+57±10 beats/min) compared with the control period (+27±6 beats/min; p<0.05), suggesting an enhanced chronotropic responsiveness to norepinephrine, consistent with the enhanced inotropic responsiveness.

Response to Phenylephrine

To establish the potential contribution of α-adrenergic stimulation to the observed responses to norepinephrine, we assessed the inotropic and pressor responses to intravenous phenylephrine. Figure 5 reveals that there was no significant increase in LV dP/dt in response to increasing doses of phenylephrine. However, a dose-related increase in mean arterial pressure was observed in each group, consistent with the anticipated vasopressor response to α-adrenergic stimulation. As has been reported previously, the baseline levels of LV dP/dt were significantly greater during developing hypertension compared with the control period. Thus, although there was the expected vasopressor responses to the α-agonist, phenylephrine, there were no detectable inotropic effects attributable to α-adrenergic stimulation.

β-Adrenergic Receptor Biochemistry

During developing hypertension, there was a significant (41%) increase in β-adrenergic receptor density (80±6 fmol/mg protein) compared with the control period (57±5 fmol/mg protein, p<0.05). During the stable hypertensive phase, β-adrenergic receptor den-
cycel activity in response to NaF (10 mM) was similar for the control period (359±21 pmol cAMP/min/mg protein), developing hypertension (323±16 pmol cAMP/min/mg protein), and stable hypertension (333±28 pmol cAMP/min/mg protein), as was the response to forskolin (0.1 mM) (control, 891±115 pmol cAMP/min/mg protein; developing hypertension, 770±72 pmol cAMP/min/mg; stable hypertension, 843±120 pmol cAMP/min/mg).

In a subset of dogs (n=5), plasma catecholamines were measured before and after the development of hypertension. There were no differences in plasma norepinephrine (control, 215±45 pg/ml; developing hypertension, 226±28 pg/ml; stable hypertension, 205±18 pg/ml) or in plasma epinephrine (control, 125±32 pg/ml; developing hypertension, 113±23 pg/ml; stable hypertension, 104±14 pg/ml). Myocardial catecholamine content was also unchanged.

**Discussion**

In the present study, we found significant changes in LV hemodynamics during the course of perinephritic hypertension. In developing hypertension (2–4 weeks), there were significant increases in end-systolic wall stress, which was normalized over time, through an increase in LV wall thickness. The increase in LV short axis diameter observed during developing hypertension returned toward the control value later during stable hypertension. Thus, although neither LV mass nor LV systolic pressure was different during developing and stable hypertension, there were important changes in LV geometry, specifically, a decrease in the ratio of cavity size to wall thickness, with the resultant normalization of end-systolic stress in the stable hypertensive phase. In addition, at baseline, we found that LV systolic function was augmented during the course of developing hypertension and was maintained at control levels later, during the course of stable perinephritic hypertension, but importantly, there was no evidence of depressed LV systolic performance.

We also observed important increases in responsiveness to intravenous norepinephrine during the development of perinephritic hypertension. Given the dynamic changes in loading conditions accompanying the evolving hypertensive process, we used the relatively load-independent index, the end-systolic stress–volume relation, to assess the LV contractile response to norepinephrine. These analyses indicated that the LV inotropic state was enhanced at baseline in developing hypertension and was maintained at control levels later, in the stable phase of hypertension. Subsequently, in response to norepinephrine, there were further shifts in the end-systolic stress–volume relation in developing hypertension compared with the normotensive control period, consistent with an enhanced inotropic response to this adrenergic agonist. Although the sensitivity and linearity of this relation and the significance of changes in the volume intercept have been questioned, the slope of the end-systolic stress–volume
relation (Ees) was clearly greater during developing hypertension compared with the normotensive control period in response to adrenergic stimulation with norepinephrine.

Given the relative insensitivity of this index to alterations in contractility,30,32 the significant increases in Ees seen in response to norepinephrine in developing hypertension were a strong endorsement of the findings of enhanced adrenergic responsiveness, independent of changes in loading conditions. To further support these findings, we observed enhanced responsiveness of the isovolumic index, LV dP/dt, to norepinephrine in developing hypertension and maintenance of the response later, during the stable phase of hypertension. Thus, by using load-dependent and load-independent indexes of LV contractility, the inotropic responses to norepinephrine were enhanced early, during developing hypertension, and maintained late in the course of perinephritic hypertension. At no time did we observe an impaired responsiveness to this adrenergic agonist.

These findings differ from the majority of previous reports,4–68–11,15 which have suggested an impairment in β-adrenergic LV contractile responsiveness in hypertension. Likely explanations for these differences stem from the use of conscious, chronically instrumented dogs in the present study and a complete characterization of the inotropic state, using an isovolumic index of contractility and the end-systolic stress–volume relation. In addition, we characterized the inotropic responses at two discrete stages in the hypertensive process and used the endogenous neurotransmitter, norepinephrine, as the adrenergic agonist. Prior studies5,6,9–11 in rats have used different hypertensive models and have used isolated papillary muscle or whole-heart preparations devoid of sympathetic innervation. Saragoca and Tarazi10 demonstrated an impaired contractile response to the intravenous administration of isoproterenol in a group of spontaneously hypertensive rats compared with controls. Gende et al11 demonstrated marked cardiac impairment to isoproterenol infusions in rats with two-kidney, one-clip Goldblatt hypertension, whereas Ayobe and Tarazi10 have noted that the impairment in β-adrenergic responsiveness is reversible with regression of hypertrophy. Tarazi12 has offered important caveats about the heterogeneity of the alteration in β-adrenergic physiology depending on whether genetic or acquired models of hypertension are used. Major species differences and model dependency make it difficult to reach a consensus on alterations in LV contractile responses to catecholamines during the development of hypertension in the rat. In addition to differences in the proportion of myocardial α- and β-receptors,16,17 there are important species differences in changes in myosin isoenzyme forms to the V1 subtype during hypertension,18 which result in slower crossbridge formation and may predominate over changes in sarcolemmal membrane receptor responses.

There have been fewer prior studies investigating alterations in adrenergic responsiveness in hypertensive canine models. Davidson et al33 suggested that β-adrenergic stimulation was maintained early (2 weeks) in the development of renovascular hypertension in dogs. Although there were some methodological differences (e.g., they conducted their studies in response to bolus infusions of isoproterenol, the animals were sedated, and changes in loading conditions were not considered), their data might really be consistent with those in the current investigation. It is important to consider that the net inotropic effect of isoproterenol is the sum of direct myocardial β-adrenergic stimulation plus reflex mediated effects. Since Davidson et al33 demonstrated depressed reflex responses, the portion of the inotropic and chronotropic responses to isoproterenol that is reflexly mediated was lost. In the absence of the reflex-mediated component, the observation that there were no differences in the inotropic response to isoproterenol implies that the direct myocardial stimulation must have been enhanced. If reflex control had been intact in these hypertensive dogs, or had the dogs been studied under ganglionic blockade, enhanced inotropic and chronotropic responses to isoproterenol might have been observed.

Thus, there are major methodological differences between prior reports in either isolated or anesthetized animal preparations and the current observations conducted in chronically instrumented dogs with perinephritic hypertension. Recent studies from our laboratory3 have underscored the importance of intact autonomic nervous system function in the enhanced LV contractile response seen at baseline in developing perinephritic hypertension. The current data extend these observations to include augmented adrenergic responsiveness to norepinephrine in developing hypertension and maintenance of a normal inotropic response to this adrenergic agonist later, when the hypertensive process has stabilized.

It is conceivable that alterations in reflex control may contribute to altered inotropic responsiveness in the intact, conscious animal.34,35 Accordingly, we investigated the responses to norepinephrine under ganglionic blockade and observed that the enhanced responses to norepinephrine persisted in developing hypertension compared with the control period. Thus, the enhanced inotropic responsiveness to norepinephrine was not abolished by ganglionic blockade, suggesting that the effects were not mediated via reflex mechanisms. The enhanced chronotropic responses to norepinephrine under ganglionic blockade are consistent with the augmented direct myocardial effects of this adrenergic agonist.

Another difference between the current investigation and most prior studies is the use of the endogenous neurotransmitter, norepinephrine, to induce adrenergic stimulation, as opposed to a specific β-adrenergic agonist.4–6,9–11,32 Thus, it is conceivable that the observed differences may be attributed to the associated α-adrenergic properties of norepineph-
rine. This explanation is unlikely in view of the virtual inability of norepinephrine to increase LV dP/dt in the presence of β-adrenergic blockade in the canine model. Similarly, we observed that there was no inotropic response to α-adrenergic stimulation in any group, suggesting that the increased inotropic responses to norepinephrine were mediated via its β-adrenergic properties.

There are several mechanisms that may account for the enhanced responses that were observed. Of obvious importance are alterations in β-adrenergic receptor biochemistry. We observed significant increases in myocardial β-adrenergic receptor density during developing hypertension, which may account for the selective increase in adrenergic myocardial responsiveness to norepinephrine. There were no significant differences in plasma or myocardial catecholamine levels for the three study periods, indicating that the changes in β-adrenergic receptor density were not due to altered levels of measurable catecholamines.

The finding of increased β-receptor density but similar maximal adenylate cyclase activity in developing hypertension provides an important and recognized method of amplification within the β-adrenergic receptor system by allowing for an increase in receptor occupancy at any given agonist concentration. A similar pattern was observed in a recent study examining mechanisms of denervation supersensitivity, which was characterized by increased β-adrenergic receptor density and no change in maximal adenylate cyclase stimulation. However, this is the first report that such mechanisms may be operative during developing hypertension, when wall stress is increased, thereby allowing for enhanced contractile responses to β-adrenergic stimulation.

The findings of increased β-adrenergic receptor density in developing hypertension are in contrast to prior reports in rats. Ayobe and Tarazi demonstrated decreases in myocardial β-receptor number in rats with renovascular hypertension and LV hypertrophy. However, Sen and Tarazi have also noted that there was marked variability in myocardial catecholamine content depending on whether genetic or acquired models of hypertension were used, even when the degree of hypertrophy was similar. Such variations likely account for the differences in findings that have been reported. For example, using a similar model, Gende et al noted depressed adrenergic responsiveness to isoproterenol but no change in β-receptor density or affinity. In their canine model of perinephritic hypertension, Davidson et al found no perturbation in the myocardial β-receptor–cyclase system but also found no evidence of hypertrophy after a brief period of hypertension. Thus, important differences in myocardial adrenergic receptor pharmacology among species, the duration of the hypertensive lesion, and the degree of associated hypertrophy may all account for the significant differences between our findings and others.

Other potential mechanisms that may help to reconcile the differences between our findings and those who found impaired adrenergic responsiveness include abnormalities in coronary flow reserve associated with hypertensive hypertrophy. Alfaro et al noted impaired coronary perfusion in rats with renovascular hypertension and associated this with impairments in contractile function. Marcus et al showed a modest decrease in coronary vasodilator reserve at 6 weeks after the development of renovascular hypertension in dogs but did not study contractile function. Furthermore, they found no further decrease in vasodilator reserve when the hypertensive lesion was allowed to persist for 6 months. As reported elsewhere, we found modest reductions in coronary vasodilator reserve in dogs with either developing or stable hypertension. However, despite this modest impairment in coronary vasodilator reserve, we observed enhanced inotropic responsiveness to norepinephrine in developing hypertension, the magnitude of which may have been even greater if coronary vasodilator reserve was not affected.

Another potential explanation for the differences between our findings and those of others pertains to alterations in myocardial structure. Another investigator has observed an increase in fibrous connective tissue in hypertensive rats and has suggested that these changes may contribute to systolic impairment in these hypertensive models. Weber et al noted an increase in the volume and a shift in the type of collagen in evolving hypertension (4 weeks) in a nonhuman primate model of perinephritic hypertension. These changes were associated with diminished systolic performance both in vivo and in vitro. In contrast, we found no significant increase in collagen content in the LV of dogs with stable hypertension, suggesting that this mechanism is not playing a role in modulating the inotropic responsiveness to catecholamines in this model. Potentially with more chronic hypertension and more severe hypertrophy, where LV fibrosis is increased substantially, this mechanism might act to decrease catecholamine responsiveness and might have been a factor in prior studies reporting depressed responsiveness to β-adrenergic stimulation.

Finally, the findings of enhanced inotropic responsiveness to norepinephrine during developing hypertension and maintenance late in the course of hypertension have important implications in the pathogenesis of the hypertensive state. Previous studies have suggested that the heart may play an important role in the development of hypertension. However, prior studies in rats have been unable to conclusively demonstrate such a role. These data demonstrate for the first time that the myocardial substrate is poised to respond to the sympathetic neurotransmitter, norepinephrine, with enhanced responsiveness early in the hypertensive state and that diminished responses to norepinephrine were not observed even during a more chronic phase of stable hypertension.

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**KEY WORDS** • systemic hypertension • left ventricular hypertrophy • contractility • β-adrenergic receptors • adenylate cyclase
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