Altered Function and Structure of the Heart in Dogs With Chronic Elevation in Plasma Norepinephrine

Mrugesh B. Patel, MD, PhD; Julian M. Stewart, MD; Alden V. Loud, PhD; Piero Anversa, MD; Jie Wang, MD; Lisa Fiegel, BA; and Thomas H. Hintze, PhD

Background. We have previously shown that chronic elevation of plasma norepinephrine leads to a functional independent increase in left ventricular weight. The goals of the present study were to determine quantitatively the component of the myocardium that accounted for the observed structural changes and to determine the function of the hypertrophied myocardium.

Methods and Results. Mongrel dogs were chronically instrumented for measurement of arterial and left ventricular pressures, left ventricular internal diameter, and left ventricular wall thickness. Subcutaneous osmotic pumps were implanted to release norepinephrine continuously for 28 days. Hemodynamics were measured with dogs in the quietly resting state and during infusions of isoproterenol at 0.1 and 0.5 μg/kg/min before and on days 14 and 28 during the infusion of norepinephrine. The hemodynamic response to 10 μg/kg phenylephrine, given as a bolus, was also assessed before norepinephrine and 28 days during the infusion of norepinephrine, and the end-systolic pressure–diameter or wall stress–diameter relations were calculated. On day 28, hearts were arrested in diastole and perfusion fixed in situ. Tissue samples were prepared for electron microscopy and morphometry. Hemodynamic studies showed that isoproterenol (0.5 μg/kg/min) reduced mean arterial pressure (MAP) to the same point on each experimental day, and the increases in indexes of contractility were reduced during norepinephrine infusion. Left ventricular dP/dt max increased 131±24% on control day, only 67±20% on day 14, and 55±18% on day 28. Similar changes were observed in dP/dt/DP 40 and dP/dt/end-diastolic circumference. However, E max, the slope of the end-systolic pressure–diameter or wall stress diameter relations, was unchanged, suggesting that inotropic state was not altered. Morphometric studies showed that the cross-sectional area of myocytes increased by 55%, but myocyte and capillary densities decreased by 34% and 29%, respectively (p<0.05) in dogs with high norepinephrine levels. There were no differences in volume fractions of myocytes, capillary lumen, or interstitium or capillary-to-myocyte ratio.

Conclusions. The myocardium of dogs with high norepinephrine levels shows reduced inotropic response to β-adrenergic stimulation despite the increases in left ventricular mass and left ventricular wall thickness, which are a result of growth of the cardiac myocytes and characteristic of concentric hypertrophy. These data suggest that chronic adrenergic stimulation of the heart reduces the β-receptor coupling to the contractile response without importantly compromising left ventricular function. (Circulation 1991;84:2091–2100)

Myocardial hypertrophy of different etiologies1–4 is associated with several pathological conditions.5–7 We have recently shown that chronic infusion of subpressor doses of norepinephrine in mongrel dogs results in increased mass of the myocardium and thickening of the left ventricular wall.8,9 Plasma norepinephrine levels in this model are similar to those seen during mitral valve prolapse,10 pheochromocytoma,11 and congestive heart failure.7 On structural analysis, pressure overload leads to concentric hypertrophy in which wall thickness increases12 through the lateral expan-
sion of myocytes with no changes in cavity volume.\textsuperscript{13,14} In contrast, volume overload is associated with lengthening of myocytes and dilation of the ventricular chamber, that is, eccentric hypertrophy.\textsuperscript{4,5,13} The mechanism by which norepinephrine induces cardiac hypertrophy is currently unknown, although it has been suggested that norepinephrine may have a trophic effect on the myocardium without affecting afterload.\textsuperscript{15,16} This possibility is consistent with Simpson's\textsuperscript{17} observation that norepinephrine induces hypertrophy in cultured neonatal rat myocytes by way of an $\alpha$-receptor--mediated mechanism. However, administration of large quantities of catecholamines can cause tissue damage or necrosis in the myocardium,\textsuperscript{18} and the remaining intact myocytes may undergo compensatory or reactive hypertrophy. Such a condition is encountered after myocardial infarction in which the remaining cells increase in length and diameter in response to the altered pre-load and afterload. The cellular growth process involved in the increase in mass of the myocardium with a chronic infusion of subpressor dose of norepinephrine has not been determined.

Early in the hypertrophic response associated with pressure and/or volume overload, the myocardium expands in mass and maintains normal function at rest and under stress.\textsuperscript{19,20} However, after a prolonged functional overload, the myocardium may maintain resting function but is not capable of handling additional stresses.\textsuperscript{21,22} With elevated plasma norepinephrine for 28 days, resting systolic and diastolic cardiac functions are preserved, and there are no signs of heart failure.\textsuperscript{8,9} Whether the hypertrophied myocardium in this setting is able to respond to additional functional loads has not been examined. Therefore, morphometric techniques were used to determine the changes in volume composition of the myocardium and shape of myocytes to establish whether the increase in mass was accomplished with an increase in myocyte cross-sectional area or length. These two types of responses have repeatedly been shown to constitute the morphological bases of pressure and volume overload hypertrophy, respectively. A primary goal of our study was to determine which components of the myocardium—myocytes, capillaries, or interstitium—are altered and, therefore, to determine the type of hypertrophy, that is, concentric or eccentric, that we observed. The second goal was to use inotropic stimulation and changes in ventricular afterload to determine whether the myocardium from dogs with chronic infusion of norepinephrine is capable of handling an additional functional load and possibly to uncover systolic or diastolic dysfunction.

**Methods**

**Surgical Procedure**

Adult mongrel dogs (20–30 kg, $n=7$) were used in this study. On the day of the surgery, dogs were weighed, tranquilized with acepromazine maleate (3 mg/kg, Tech America), and anesthetized with an intravenous injection of 25 mg/kg sodium pentobarbitol (Butler). An endotracheal tube was inserted and connected to a respirator (Harvard Apparatus). Surgery was performed using sterile surgical techniques. The thorax was opened by means of a left lateral thoracotomy at the fifth intercostal space. Tygon catheters (Norton Plastics) were inserted in the descending aorta and the left atrial appendage. A solid-state pressure gauge (Konigsberg, p 6.5) was inserted in the left ventricle at the apex. A pair of 3-MHz piezoelectric crystals were placed on opposing endocardial surfaces at the base of the left ventricle. A second pair of sonomicrometers (7-MHz piezoelectric crystals) was placed in the anterior wall at the base of the left ventricle for measurement of wall thickness. The wires and catheters were exteriorized between the scapulae, and the chest was closed in layers. The pneumothorax was reduced, and the dogs were allowed to recover fully.

Antibiotics were administered after surgery. Heart rate and temperature were monitored daily, and body weight was monitored on alternate days. After 10 days, dogs were trained to lie quietly on the laboratory table. (We have used these techniques previously.\textsuperscript{8,23–25})

**Experimental Protocol**

Cardiac functions were continuously recorded with dogs in quietly resting conditions and during infusions of 0.1 and 0.5 $\mu$g/kg/min isoproterenol and 10 $\mu$g/kg phenylephrine given as a bolus injection. At each dose, isoproterenol was infused until a steady-state response was obtained. The infusions lasted 7–8 minutes, and data were sampled after 5–6 minutes. The dose of phenylephrine was chosen to increase systolic arterial pressure by 30–40 mm Hg. The sequence of isoproterenol infusions and phenylephrine injection was chosen randomly, and cardiac functions were allowed to return to preinfusion values after each dose. Cardiac functions were recorded on a 14-channel magnetic tape (Bell and Howell, 3700B) and printed on a direct writing oscillograph (Gould-Brush, 2800s). Following this protocol, osmotic infusion pumps (Alza, 2mL4) were implanted subcutaneously to continuously release norepinephrine at 0.5 $\mu$g/kg/min for 28 days, and the experimental protocol was repeated on days 14 and 28 of norepinephrine infusion.

**Perfusion Fixation**

After 28 days, hearts were perfusion fixed in situ in six normal dogs and six dogs with norepinephrine infusion. The dogs were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and mechanically ventilated. The thorax was opened in the left fourth intercostal space. A catheter was placed in the left ventricle through a stab wound. Heparin (3,000 units, Lyphomed) was injected intravenously, and the heart was arrested with a bolus injection of saturated potassium chloride. The aorta was cannulated for retrograde perfusion, and the right atrial appendage was cut to allow outflow of perfusate. The heart was
perfused with 1,500 ml of 0.1 M phosphate buffer. The pressure in the left ventricular cavity was fixed at the measured in vivo end-diastolic pressure, approximately 8 mm Hg through the catheter, and the heart was perfused with 1,200 ml of freshly made solution of 2.5% glutaraldehyde and 2% paraformaldehyde. The perfusion-fixed heart was excised, and the ventricular tissue was isolated and cut to separate the left ventricle, the right ventricle, and the septum, which were weighed. Wall thickness in the left ventricle at the base was measured using planimetry (Zeiss Videoplan). (We have used these techniques previously.) Tissue from subendocardium of the anterior wall of the left ventricle at the base of the heart, where calculated diastolic wall stress is maximal, was sampled for microscopic studies.

**Tissue Preparation for Electron Microscopy**

Fifteen samples, 1–2-mm cubes, were taken from the subendocardial region of the anterior wall of the left ventricle at the base. The sections were cut perpendicular to the long axis of myocytes and capillaries or parallel to the minor axis of the left ventricular cavity. The tissue was prepared and embedded in araldite plastic (Fluka, Switzerland). One-micrometer-thick sections of the embedded tissue were stained with toluidine blue and used for measurement of sarcomere lengths. Fifty-nanometer-thick sections of selected regions (0.3×0.3 mm) were prepared and photographed at ×1,600 with a calibrated magnification standard using a Siemens 101 electron microscope. The final electron micrographs were printed at ×5,000 magnification, overlaid with a 13×17 morphometric grid. Morphometric techniques were used to measure three components of the myocardium: myocytes, capillaries, and the interstitium. The measured parameters included volume fractions of myocytes, myocyte nuclei, capillary lumen, capillary endothelium, and interstitium; average cross-sectional area of myocytes and capillary lumen; and surface area of capillaries and capillary length. The numbers per unit area of capillaries and myocytes were counted using the “forbidden line” rule. The person performing the counting was blinded from the identity of the tissue, that is, whether tissue was from a normal dog or one infused with norepinephrine, until the analysis of all the hearts was complete. Therefore, the morphometric analysis was independent of the gross weight analysis of the hearts.

**Hemodynamics**

Arterial pressure was measured using the fluid-filled catheter in the aorta and a strain gauge pressure transducer (Statham, P23ID). Mean arterial pressure (MAP) was derived using resistance-capacitance filters with a 2-second time constant. Left ventricular pressure (LVP) was measured using a solid-state pressure gauge (Koningsberg, p 6.5). The first derivative of LVP (LV dP/dt) was obtained using operational amplifiers (National Semiconductor LM324). Maximum (+)LV dP/dt was examined as an index of LV contractility, and maximum (−)LV dP/dt was examined as an index of LV relaxation during isoproterenol infusion. LV dP/dt at a developed pressure of 40 mm Hg (LV dP/dt/DPo) and LV dP/dt/end-diastolic circumference (LV dP/dt/EDC) were used as normalized indexes of contractility during isoproterenol infusion.24,25,26 Heart rate was obtained using a cardiotachometer (Beckman, 9857B). LV internal diameter and LV wall thickness were measured by sonomicrometers with a Transit Time dimension gauge.

Pressure–diameter loops were constructed for the left ventricle by playing back data recorded during the phenylephrine injection, converting those data into digital records, and then simultaneously plotting LV pressure and LV diameter (Gould Electronics, DASA system), thereby obtaining a parametric plot of pressure versus diameter. The LV end-systolic pressure–diameter relation was obtained following the methods of Sagawa et al. and linear least squares methods were used to obtain the slope of the pressure–diameter relation (Emax), which was used as an index of LV contractility and the x intercept of that relation, which represents the unstressed cardiac diameter (D0). To normalize for changing wall thickness during norepinephrine infusion, pressure–diameter data were transformed into wall stress–diameter data by using the formula of Sasayama et al., (σ=P·D/[4·thickness]) to estimate wall stress, where P is pressure and D is diameter. Shortening was measured as the difference between end-diastolic diameter (EDD) and end-systolic diameter (ESD), and percent shortening was calculated as (EDD−ESD/EDD)×100. Wall thickening was measured as the difference between end-diastolic and the end-systolic wall thickness (DWT and SWT, respectively). Percent wall thickening was calculated as (SWT−DWT/DWT)×100. Time in diastole per beat (TDB) was measured from closing of the aortic valve, which is the minimum internal diameter, to beginning of the upstroke of the dP/dt signal, which is the beginning of systole. Time in diastole per minute (TDM) was calculated as a product of TDB and HR.

**Statistics**

Comparisons of hemodynamic data in conscious dogs were made between the baseline and response to isoproterenol on each experimental day and between the responses to isoproterenol on the control day compared with 14 and 28 days during norepinephrine, using two-way analysis of variance. Emax and D0 were calculated for every dog on the control day and on day 28. Data on these days were compared by unpaired t test. The planimeted data for internal diameter and wall thickness, as well as heart weights, were compared using the unpaired Student’s t test. The morphometric data in the two groups were compared using group t test. The slopes of the end-systolic pressure–diameter or wall stress–diameter relation were calculated using a linear least
TABLE 1. Contractile Response During Infusions of Isoproterenol

<table>
<thead>
<tr>
<th>Isoproterenol</th>
<th>Control day</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Response</td>
<td>Baseline</td>
</tr>
<tr>
<td>Peak left ventricular pressure (mm Hg)</td>
<td>131±3.2</td>
<td>134±3.9</td>
<td>129±1.4</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>132±2.5</td>
<td>137±2.8</td>
<td>129±3.6</td>
</tr>
<tr>
<td>Left ventricular dP/dt (mm Hg/sec)</td>
<td>3,032±126</td>
<td>4,864±435*</td>
<td>3,071±149</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>3,154±132</td>
<td>7,158±674*</td>
<td>3,046±133</td>
</tr>
<tr>
<td>Left ventricular dP/dt/EDC (1/sec)</td>
<td>62.3±3.0</td>
<td>92.6±9.6*</td>
<td>62.5±5.5</td>
</tr>
<tr>
<td>0.5 µg/kg/min</td>
<td>64.1±4.0</td>
<td>121±15*</td>
<td>63.3±4.2</td>
</tr>
<tr>
<td>Left ventricular dP/dt/EDC (mm Hg/sec/mm)</td>
<td>24.5±1.3</td>
<td>41.7±5.2*</td>
<td>25.1±1.6</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>25.5±1.7</td>
<td>63.7±7.0*</td>
<td>25.0±1.4</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>32.6±1.5</td>
<td>30.1±1.4*</td>
<td>30.8±1.6</td>
</tr>
<tr>
<td>0.5 µg/kg/min</td>
<td>32.4±1.4</td>
<td>28.3±1.3*</td>
<td>30.7±1.5</td>
</tr>
<tr>
<td>Shortening (mm)</td>
<td>7.7±0.7</td>
<td>8.5±0.5</td>
<td>9.2±0.4</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>8.2±0.7</td>
<td>9.2±1.1*</td>
<td>9.1±0.5</td>
</tr>
<tr>
<td>Shortening (%)</td>
<td>18.6±1.3</td>
<td>21.3±1.0*</td>
<td>22.4±1.1</td>
</tr>
<tr>
<td>0.5 µg/kg/min</td>
<td>19.2±1.2</td>
<td>21.8±1.6*</td>
<td>22.0±0.9†</td>
</tr>
<tr>
<td>Systolic wall thickness (mm)</td>
<td>14.2±0.2</td>
<td>16.0±0.1*</td>
<td>16.2±0.1†</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>14.2±0.3</td>
<td>17.0±0.2*</td>
<td>16.0±0.2*</td>
</tr>
<tr>
<td>Wall thickening (mm)</td>
<td>2.8±0.34</td>
<td>3.4±0.24*</td>
<td>3.9±0.64</td>
</tr>
<tr>
<td>0.5 µg/kg/min</td>
<td>2.9±0.26</td>
<td>3.3±0.11*</td>
<td>4.0±0.51</td>
</tr>
<tr>
<td>Wall thickening (%)</td>
<td>24.2±3.0</td>
<td>0.3±3.6*</td>
<td>26.4±2.7</td>
</tr>
<tr>
<td>0.1 µg/kg/min</td>
<td>23.8±2.0</td>
<td>30.0±3.4*</td>
<td>24.8±3.1</td>
</tr>
<tr>
<td>0.5 µg/kg/min</td>
<td>22.8±2.0</td>
<td>30.0±3.4*</td>
<td>24.8±3.1</td>
</tr>
</tbody>
</table>

*p<0.05 from baseline.
†p<0.05 from control day.

Results

Seven conscious dogs received isoproterenol on the control day and 14 and 28 days during continuous infusion of norepinephrine. Five of these dogs also received phenylephrine on the control day and on day 28.

Baseline Hemodynamics

Tables 1 and 2 list cardiac functions in the seven dogs in the quietly resting state. There was a significant reduction in HR on days 14 and 28 compared with the control day. No changes were observed in MAP, LVP, LV dP/dt max, LV dP/dt/DP ao, LV dP/dt/EDC, end-diastolic dimension (EDD), or end-systolic dimension (ESD), whereas percent shortening was increased on days 14 and 28. The increase in diastolic wall thickness was consistent with the observed hypertrophy. Maximum (−)dP/dt, an index of relaxation, was significantly reduced on day 28. The observed increase in TDB is consistent with the reduction in HR on days 14 and 28.

Effects of Isoproterenol Infusion

Table 1 shows the responses to isoproterenol infusions at 0.1 and 0.5 µg/kg/min; only the 0.1-µg/kg/min dosage will be discussed in the text. At this dosage of isoproterenol, the increase in dP/dt max from baseline was significantly reduced on days 14 (25±5.0%) and 28 (40±10%) compared with that on the control day (62±16%). dP/dt/DP ao, an afterload-independent index, and dP/dt/EDC, a preload-inde-
Table 2. Diastolic Functions During Isoproterenol Infusion

<table>
<thead>
<tr>
<th>Isoproterenol</th>
<th>Control day</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Response</td>
<td>Baseline</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>104±3.0</td>
<td>93±3.2*</td>
<td>104±2.4</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>107±2.8</td>
<td>93±4.3*</td>
<td>101±2.6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>94±2.4</td>
<td>148±10*</td>
<td>63±1.3†</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>89±3.9</td>
<td>193±0.15*</td>
<td>67±3.7†</td>
</tr>
<tr>
<td>(-)dp/dt max (mm Hg/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>3,076±118</td>
<td>3,287±209</td>
<td>2,944±110</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>3,106±81</td>
<td>3,510±308</td>
<td>3,001±112</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>40.0±1.7</td>
<td>38.2±1.6*</td>
<td>39.5±1.7</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>40.2±1.8</td>
<td>36.7±1.6*</td>
<td>39.2±1.6</td>
</tr>
<tr>
<td>Diastolic wall thickness (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>11.4±0.3</td>
<td>12.4±0.3</td>
<td>12.9±0.2†</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>11.3±0.2</td>
<td>12.9±0.2*</td>
<td>13.0±0.2†</td>
</tr>
<tr>
<td>Time in diastole per beat (seconds)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>0.50±0.02</td>
<td>0.28±0.02*</td>
<td>0.75±0.03†</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>0.52±0.03</td>
<td>0.19±0.02*</td>
<td>0.75±0.03†</td>
</tr>
<tr>
<td>Time in diastole per minute (seconds)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μg/kg/min</td>
<td>46.9±1.3</td>
<td>41.3±1.5*</td>
<td>47.4±1.2</td>
</tr>
<tr>
<td>0.5 μg/kg/min</td>
<td>45.5±1.1</td>
<td>35.2±2.7*</td>
<td>49.1±1.4†</td>
</tr>
</tbody>
</table>

*p<0.05 from baseline.
†p<0.05 from control day.

Effects of Phenylephrine on Pressure–Diameter Relation

Table 3 shows the hemodynamic effect of 10 μg/kg phenylephrine on the control day and on day 28 of norepinephrine infusion. MAP, LV systolic and end-diastolic pressures, and LV end-diastolic and end-systolic diameters increased, whereas heart rate decreased by similar amounts before (control) and after (day 28) norepinephrine had been infused. Representative end-systolic pressure–diameter linear regression lines are shown for a dog in Figure 2. Table 4 shows the calculated E_max and D_0 data obtained from pressure-diameter loops on the control day and on day 28. There was no significant difference between E_max and D_0 on the control day and on day 28 during the norepinephrine infusion. Calculation of end-systolic wall stress–diameter relations and E_max for these also showed no significant effect of norepinephrine infusion (Table 4).

Gross Measurements

Heart weights and body weights were measured in six normal dogs and six dogs with high norepinephrine levels for 28 days. In dogs with high norepinephrine levels, significant increases were found in gross weights of the left ventricle (61.2%), right ventricle (67.1%), and the septum (65.1%). When normalized to body weight, there were significant increases found in ratios of left ventricle to body weight (50.6%), right ventricle to body weight (55.6%), and septum to body weight (53.9%). Diastolic wall thickness, measured by planimetry, was 22.8% larger in the high-norepinephrine group than in the normal dogs (Figure 3). Wall thickness measurements by planimetry are also similar to the values obtained by sonomicrometers in the conscious dogs on control day and on day 28 of norepinephrine infusion.

Morphometric Analysis

Morphometric analysis was performed on tissues from six normal dogs and six dogs that had received norepinephrine for 28 days. There was no difference in sarcomere lengths between normal dogs and dogs with elevated norepinephrine. The volume fractions of cardiac myocytes, myocyte nuclei, capillary lumen, capillary endothelium, and the inter-

Dependent index, were similarly reduced on days 14 and 28 compared with control. On each experimental day, this dose of isoproterenol increased heart rate by the same amount. However, because of the bradycardia, which occurred in dogs in the quietly resting state during norepinephrine infusion, the changes from baseline on days 14 (166±26%) and 28 (112±15%) were significantly larger than on control day (58±9.5%). Similar changes in heart rate and dp/dt (Figure 1) were seen in response to the larger dosage of isoproterenol. For each infusion rate, the changes in MAP, EDD, DWT, TDB, and TDM on days 14 and 28 were similar to those on control day (Table 2). There were no significant changes in LVP.
with an increase in diffusion distance for oxygen.\textsuperscript{1,13} This contrasts with the characteristic changes of eccentric hypertrophy that occur with chronic volume overload and are due primarily to elongation of cardiac myocytes and preservation of capillary concentration in the myocardium.\textsuperscript{35,36}

Chronic infusion of norepinephrine in mongrel dogs causes structural alterations that share aspects of both concentric and eccentric types of hypertrophy despite the absence of either pressure or volume overload. As in concentric hypertrophy, the wall of the left ventricle is thickened uniformly from base to apex. The LV wall at the base, where tissue samples were obtained for microscopic studies, was 22.8\% thicker in diastole compared with that in normal dogs using morphometric measurements and 20.8\% thicker using sonomicrometric measurements. Morphometrically, the cross-sectional area of myocytes increased 51\%, which is equal to a 23\% increase in cell diameter and almost identical to the magnitude of the thickening of the LV wall. Furthermore, no significant increase in the volume fraction of the interstitium occurred, supporting our conclusion that cell loss and myocardial fibrosis did not take place with chronic elevation of plasma norepinephrine levels for 28 days in dogs.

We found that the volume fraction of cross-sectional area of the capillary lumen did not change with norepinephrine-induced hypertrophy, whereas capillary density decreased due to hypertrophy of the myocytes. There were no significant differences in

\textbf{Discussion}

Cardiac hypertrophy occurs in response to chronic pressure and volume overloads,\textsuperscript{4,13} during development,\textsuperscript{1} with exercise training,\textsuperscript{32,33} and in association with endocrine factors\textsuperscript{34} such as thyroid hormone and growth hormone. Each of these stimuli induces specific gross and structural changes in the myocardium. Concentric hypertrophy occurs with pressure overload\textsuperscript{5,12} and consists of an increase in myocyte cross-sectional area and a reduction in capillary density, stitium were not different. In the dogs given norepinephrine, cross-sectional area (\textmu{}m\textsuperscript{2}) of myocytes increased by 55\% (Figure 4), whereas the cross-sectional area of the capillary lumen did not change compared to normal dogs. Figure 5 shows that there was a significant reduction in myocyte density (34\%) and capillary density (29\%) in the high-norepinephrine group. The capillary-to-myocyte ratios were not different in the two groups. Diffusion distance was increased in dogs with high norepinephrine by 30\%. Surface area of capillaries as measured over a volume of myocytes or volume of myocardium was significantly reduced (41\% and 38\%, respectively).

\begin{table}
\centering
\begin{tabular}{|l|l|l|}
\hline
 & Control & Change from control \\
\hline
Mean arterial pressure (mm Hg) & & \\
Before norepinephrine & 108±6 & 56±10\* \\
Day 28 & 121±4.2 & 76±11\* \\
Heart rate (beats/min) & & \\
Before norepinephrine & 95±10 & -27±6.5\* \\
Day 28 & 75±3 & -13±6.0\* \\
Left ventricular systolic pressure (mm Hg) & & \\
Before norepinephrine & 135±8.4 & 56±6\* \\
Day 28 & 142±3.3 & 83±13\* \\
Left ventricular end-diastolic pressure (mm Hg) & & \\
Before norepinephrine & 7.0±0.5 & 10±1.1\* \\
Day 28 & 5.8±0.3 & 9.0±3.7\* \\
Left ventricular end-diastolic diameter (mm) & & \\
Before norepinephrine & 36±1.7 & 0.8±0.2\* \\
Day 28 & 37±1.1 & 1.8±0.1\* \\
Left ventricular end-systolic diameter (mm) & & \\
Before norepinephrine & 29±1.7 & 2.3±0.5\* \\
Day 28 & 29±1.6 & 2.5±0.2\* \\
\hline
\end{tabular}
\caption{Hemodynamic Effects of Phenylephrine (10 \textmu{}g/kg) Before and After 28 Days of Norepinephrine Infusion}
\end{table}

\*p<0.05 from control.
TABLE 4. $E_{\text{max}}$ and $D_0$ Data After Phenylephrine

<table>
<thead>
<tr>
<th></th>
<th>Control day</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure–diameter loops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>24.8±2.3</td>
<td>25.0±2.0</td>
</tr>
<tr>
<td>$D_0$</td>
<td>26.8±2.1</td>
<td>24.8±1.9</td>
</tr>
<tr>
<td>Wall stress–diameter loops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>23.5±2.3</td>
<td>23.5±2.6</td>
</tr>
<tr>
<td>$D_0$</td>
<td>28.0±1.9</td>
<td>26.8±2.0</td>
</tr>
</tbody>
</table>

$E_{\text{max}}$, Slope of pressure–diameter relation; $D_0$, x intercept of $E_{\text{max}}$. 

Additional functional loads when oxygen consumption increases dramatically. Once myocardial hypertrophy has been established, it can be characterized in terms of functional reserve.

We have previously reported the effects of infusion of norepinephrine at 0.5 μg/kg/min for 28 days in chronically instrumented mongrel dogs. In brief, plasma levels of norepinephrine increased from 238 pg/ml before infusion and remained elevated between 3,000 and 4,000 pg/ml throughout the period of infusion, whereas heart rate and cardiac output in the quietly resting state decreased and then remained constant throughout the 28 days. Resting preload, afterload, indexes of contractility, calculated stroke work and minute work, and indexes of myocardial oxygen consumption were unaffected by norepinephrine administration. These observations were
confirmed in the present study. There were no signs of heart failure such as increased LV end-diastolic pressure, reduced indexes of contractile state, tachypnea, edema, ascites, or electrocardiographic changes.39

To uncover the potential for systolic and diastolic dysfunction and determine the functional reserve of the left ventricle, inotropic state was altered by infusing isoproterenol. On control day, 0.1 μg/kg/min isoproterenol caused significant increases from baseline in heart rate, LV dP/dt, and percent wall thickening. SWT increased from 14.2±0.2 to 16±0.1 mm (p<0.05). Wall thickening increased from 24.2±3.0% to 30.3±3.6% (p<0.05). Infusion of 0.5 μg/kg/min of isoproterenol increased LV dP/dt despite the reduction in preload, and the change was larger than that seen with low-dose isoproterenol. Percent shortening and percent wall thickening were significantly increased from baseline. These results in normal dogs are similar to those seen by Vatner et al.40 In that study, isoproterenol infusion increased DWT from 11.4±0.3 to 12.4±0.3 mm and SWT from 14.2±0.2 to 17.0±0.2 mm. Percent wall thickening was also elevated. Similar cardiac function and wall thickness measurements were obtained by Gallagher et al.,41 who infused isoproterenol at 0.4 μg/kg/min in chronically instrumented conscious dogs.

During chronic infusion of norepinephrine, both doses of isoproterenol reduced MAP from baseline, and the reduction on each experimental day was similar to that on the control day. Heart rate increased from baseline with both doses of isoproterenol on each of the experimental days. The changes in heart rate were not different from those on control day. The increase in heart rate may be a direct effect of isoproterenol on the sinoatrial node via the β-adrenergic receptors; heart rate may also be elevated in response to unloading of the systemic arterial baroreceptor reflex during reductions in MAP. The baroreflex response consists of withdrawal of vagal tone and activation of the sympathetic input to the heart, which mediates increases in heart rate indirectly via β₁-adrenergic receptors.

The magnitude of the contractile response to isoproterenol was, however, considerably reduced during norepinephrine infusion compared with the control day. Thus, with the higher dosage, maximum LV dP/dt, dP/dt/Dp40, dP/dt/EDC, percent shortening, and percent wall thickening all increased from baseline on each of the experimental days, but the increases were smaller than those on control day. In contrast, our assessment of inotropic state as measured by the end-systolic pressure–diameter or stress–diameter relations suggests that no intrinsic change occurs in the contractile apparatus, resulting in no significant change in Eₘₐₓ or in D⁰.

Our technique of using bolus injections of phenylephrine to increase arterial pressure and construct the pressure–volume relation from which we calculate Eₘₐₓ may have some constraints. First, these are not steady-state values; instead, the data are sampled while pressure is increasing. During this time, a bradycardia, most likely vagal, was also observed. It is possible that additional vagal restraint could alter myocardial function through a force–frequency relation or a negative inotropic action of acetylcholine. The Treppe phenomenon has a very weak inotropic effect in the awake dog, and, in general, vagal innervation of the myocardium below the atroventricular node is sparse and not thought to have major influences on inotropic state. Furthermore, our studies were longitudinal, and each dog was studied and served as its own control; therefore, the differences in Eₘₐₓ are based on the same technique on a previous day in the same dog. Using bolus injections of phenylephrine, we recently found (unpublished observations) a marked depression in the calculated Eₘₐₓ using chronic pacing to induce heart failure in awake dogs. Be that as it may, we recognize the limitations of using Eₘₐₓ or any other measure alone as an unequivocal index of contractile state.52,43

Receptor binding studies indicate that the number of β-adrenergic receptors and their binding affinities are unchanged in dogs with 10–15-fold increases in plasma norepinephrine levels for 28 days,44,45 whereas there is specific uncoupling of cardiac β-receptors from adenylate cyclase. Alternatively, a reduction in inotropic reserve could represent a true change in contractile performance caused by insufficient oxygen delivery as suggested by Vatner et al.40 and Murray and Vatner,21 or could be due to structural changes in contractile proteins, especially myosin ATPase.20 A reduction in inotropic reserve is unlikely because isoproterenol increased LV dP/dt by only 100%, whereas exercise can increase contractile state by 500%. Therefore, in our study, isoproterenol infusion is a moderate inotropic stimulus and would not test contractile reserve unless it was dramatically reduced. In spontaneously hypertensive rats with hypertrophy, there is a reduction in adrenergic receptor density with no
changes in adenylate cyclase activity, whereas rats with renal hypertension have increased β-adrenergic receptors and reduced adenylate cyclase activity due to a defect in a coupling protein.46 A structurally altered myosin ATPase is found in hypertrophied hearts compared with normal hearts in the rat.19,47,48 With persistent overloads, the slower and more efficient enzyme is expressed and is apparently unable to maintain appropriate cardiac function.20,22 This is unlikely in the dog because the dominant myosin ATPase is already type 3, and in preliminary studies we have found no increase in type 3 myosin ATPase (unpublished results) in dogs with chronically elevated plasma norepinephrine. Furthermore, we have found no consistent functional overload in dogs with chronic norepinephrine infusion.

The apparent discrepancy between infusions of isoproterenol and the use of the end-systolic pressure–diameter or stress–diameter relations in the evaluation of inotropic state in dogs with chronic norepinephrine infusion is a result of where each of these probes works in the sequence of activation of contraction. Isoproterenol activates the contractile apparatus through β adrenergic receptor coupling to cyclic AMP and the contractile apparatus. In contrast, an increase in afterload alters the position on a Frank-Starling curve to generate an end-systolic pressure–diameter relation. Thus, in disease states where the β-adrenergic receptor, for example, or the coupling of a receptor to the contractile apparatus may be altered, a change in receptor-mediated contractile function may not reflect changes in inotropic state but instead altered receptor mechanisms. Our use of the E_max relation to evaluate inotropic state obviates using a receptor-linked event. This method, that is, the combined use of β-adrenergic stimulation and the pressure–diameter relation in hearts in intact animals, may allow the dissociation of receptor-mediated dysfunction from contractile dysfunction in vivo.

In conclusion, chronic infusion of a subpressor dosage of norepinephrine causes myocardial hypertrophy that is due to hypertrophy of the myocytes without growth of the capillaries or increase in the connective tissue component. The hypertrophied myocardium shows normal function at rest and normal E_max and D_0 but has reduced inotropic response to β-adrenergic receptor stimulation, suggesting reduced functional reserve that we believe is caused by an uncoupling of β-receptors, not reduced LV contractile state.

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KEY WORDS • E_max • wall thickness • isoproterenol • interstitium
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