Effects of Chronic Progressive Myocardial Hypertrophy on Indexes of Cardiac Autonomic Innervation

Klaus Lindpaintner, Donald D. Lund, and Phillip G. Schmid

The development of cardiac hypertrophy is associated with marked changes in cardiac autonomic innervation. Significant and sustained reductions of myocardial catecholamine stores and activities of tyrosine hydroxylase and dopamine β-hydroxylase have been reported in models of acutely induced ventricular hypertrophy. Conversely, activity of choline acetyltransferase, a marker of parasympathetic nervous function, shows transient increases during the development of acute right ventricular hypertrophy. The potential physiological importance of these changes prompted us to examine a clinically more relevant model of slowly progressive ventricular hypertrophy. Application of a loose band around the pulmonary artery of weanling guinea pigs resulted in a growth-related progressive right ventricular pressure overload. Right ventricular weight-to-body-weight ratio was increased significantly and progressively at 9 and 18 weeks in banded animals (0.92 ± 0.05 and 1.31 ± 0.11 mg/g, respectively, p<0.01) compared with sham-operated controls (0.55 ± 0.02 and 0.59 ± 0.01 mg/g, respectively) but showed no further gain at 27 weeks (1.41 ± 0.10 mg/g). Activities of tyrosine hydroxylase and dopamine β-hydroxylase remained unchanged in all experiment groups, while right ventricular contents of norepinephrine in banded animals at 18 and 27 weeks exhibited sustained and progressive increases (2.45 ± 0.11 and 3.40 ± 0.19 μg/right ventricle, respectively) over controls (1.80 ± 0.13 and 2.40 ± 0.22 μg/right ventricle, respectively, p<0.01). The activity of choline acetyltransferase was markedly elevated in banded animals at 18 weeks (32.6 ± 2.7 nmol/hr/right ventricle) but returned to baseline by 27 weeks (22.8 ± 1.4 nmol/hr/right ventricle). We conclude that the time course of pressure overload is an important variable influencing sympathetic neural indexes in the hypertrophied heart. Thus, in contrast to acutely induced right ventricular hypertrophy, markers of sympathetic innervation may be preserved in severe cardiac hypertrophy provided it develops in a physiological, gradual fashion. On the other hand, transient increases of the cardiac parasympathetic marker appear to be a common feature of the development of myocardial hypertrophy produced by either acute or chronic progressive pressure overload. Our observations suggest that sympathetic-parasympathetic interactions may vary during the development of chronic progressive cardiac hypertrophy and that these interactions may differ in chronic progressive and acute ventricular hypertrophy. (Circulation Research 1987;61:55–62)

Profound changes in cardiac autonomic innervation accompany the imposition of increased load on the heart. Depletion of sympathetic neurotransmitter stores is a well-recognized phenomenon in congestive heart failure in humans and experiment animals. Similarly, loss of myocardial catecholamines commonly is seen in the absence of failure in models of cardiac hypertrophy induced by constriction of aorta or pulmonary artery (or systemic hypertension). These findings have been linked to reductions in the activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthetic pathway for catecholamines, and to a defect in the reuptake mechanism at the presynaptic membrane. Recently, it has been shown that in cardiac hypertrophy, marked reductions in the ventricular activities of tyrosine hydroxylase and dopamine β-hydroxylase also occur in the absence of appreciable changes of norepinephrine content. The suggested dependence of the hypertrophied heart on adrenergic support as well as the proposed role of catecholamines in the pathogenesis of cardiac hypertrophy indicate that changes in autonomic cardiac innervation may carry great physiologic and pathologic importance.

Most of these studies were performed in models in which cardiac hypertrophy was induced acutely. In contrast, the spontaneously hypertensive rat and the cardiomyopathic Syrian hamster (during its compensated stage) do not display a reduction in indexes of cardiac sympathetic function. In both models, cardiac hypertrophy develops over a considerably longer time span than in the great majority of other experiment models. The time course over which hypertrophy develops significantly influences a number of biochemical parameters in the hypertrophying heart.
thus, it also may be an important determinant of changes in cardiac autonomic innervation in cardiac hypertrophy.

By modifying an experiment preparation of acute right ventricular pressure overload in which we have previously demonstrated profound alterations of indexes of sympathetic innervation, a model of chronically progressive right ventricular hypertrophy was developed. Thus, we were able to examine, in a clinically and physiologically relevant form of cardiac hypertrophy, whether temporal dynamics rather than the absolute magnitude of cardiac mass determine the fate of sympathetic nervous markers. In addition, the activity of choline acetyltransferase, a marker of parasympathetic nervous function in this model, was measured.

Materials and Methods

Preparation of Experiment Animals
Male weanling guinea pigs weighing 240-300 g were used in all experiments. Under sodium pentobarbital anesthesia (31 mg/kg ip), animals were intubated endotracheally and ventilated with a Harvard Rodent Respirator. The intrathoracic cavity was entered by lateral anesthesia (31 mg/kg ip), animals were intubated through the left second intercostal space, and the pulmonary artery was isolated by blunt dissection in 1 group of animals (banded), a 1.9 mm (i.d.) band was applied around the pulmonary artery just proximal to its bifurcation. In a second group (sham-operated), a suture was placed temporarily around the pulmonary artery. After closure of the thoracotomy and evacuation of the pleural space, animals were allowed to recover. Groups of 3 randomly selected banded or sham-operated animals then were housed in cages where they had free access to tap water and regular guinea pig diet (Teklad, Winfield, Iowa) killed by cervical dislocation at 9, 18, and 27 weeks after surgery. Thus, the protocol consisted of 6 experiment groups: 3 banded (B9, B18, and B27) and 3 sham-operated (S9, S18, and S27) groups.

Analytical Methods

Tissue Preparation. The heart was quickly removed, blotted, and dissected into right and left atrium, right and left ventricular free wall, and septum. Dissection of all hearts was performed by the same investigator, who was unaware of the experiment group. Samples were weighed to the nearest milligram on a Mettler H 30 balance, immediately frozen in liquid nitrogen, and stored at -70 ° C until analysis. Subsequently, samples were processed in batches of 1 tissue from all groups. Samples were thawed on ice and homogenized with 2 10-second bursts of a Tekmar Tissumizer (Cincinatti, Ohio) in 20 volumes of ice-cold 5 mM potassium phosphate and 0.1 mM ethylenediaminetetraacetic acid (EDTA) containing 0.02% Triton X-100, to which the internal standard, dihydroxybenzylamine (DHBA) (100 ng/ml), was added simultaneously with the tissue.

Norepinephrine Determinations. Immediately after homogenization, 1.5 N perchloric acid was added at a ratio of 1:2 to an aliquot of the homogenate and centrifuged at 12,000g for 5 minutes at 4 ° C. To extract catecholamines from the supernatant, a modification of the alumina adsorption technique at pH 8.5 and eluted with 0.05 N acetic acid was used. Catecholamines were isolated from these extracts by high pressure liquid chromatography using a Millipore-Waters Novapak C18 reverse phase column (μ particle size, Milford, Mass.) and a Waters U6K injector. The isocratic mobile phase, consisting of 0.15 M monochloroacetic acid containing 2 mM EDTA and 0.13 mM sodium octyl sulfate, was pumped by a Waters 6000A pump at a rate of 3.2 ml/min. A Bioanalytical Systems amperometric electrochemical detection system (West Lafayette, Ind.) with the detector potential set at +0.75 V against the Ag/AgCl reference electrode was used for quantification of norepinephrine. A Hewlett-Packard 3390A Integrator served to record and integrate the signals from the amperometric cell. Fresh external standards were prepared each day for norepinephrine and DHBA. In preliminary experiments, a close correlation between recovery of the internal standard and tissue catecholamines had been established. Tissue norepinephrine contents were calculated according to the recovery of the internal standard for each sample separately.

Determination of Enzyme Activities. The method of Coyle was employed for tyrosine hydroxylase determination, using a 10-minute incubation period at 37 ° C for a 50 μl aliquot of each tissue sample. With guinea pig heart, the rate was linear over 20 minutes and proportional to the amount of protein between protein concentrations of 1 and 5 mg/ml. The final concentrations of tyrosine and 2-aminooxyhydroxy-6,7-(dimethyltetrahydro)pteridine (DMPH) were 0.2 and 1.0 mM, respectively. The procedure of Coyle and Axelrod was used to measure activity of dopamine β-hydroxylase, using a 30-minute incubation at 37 ° C. Choline acetyltransferase activity was determined during a 15-minute incubation period as described previously by Roskoski et al. Hemodynamic Measurements. In separate groups of banded and sham-operated animals, which were not used for biochemical assays, right ventricular pressures at 27 weeks were measured. Animals were anesthetized, intubated, and ventilated. A cannula was inserted into the right ventricle through a right thoracotomy. Heart rate and pressures were recorded using a P50 Gould pressure transducer and a Gould biotachometer and pressure processor in line with a Gould 2600S Recorder, Cleveland, Ohio.

Statistical Analysis. All data are expressed as mean ± SEM. For comparisons of cardiac mass, absolute as well as relative weights were used by calculating the ratio of ventricular weight to body weight at sacrifice. Enzyme activities and norepinephrine values were expressed per gram of tissue as well as, where appropriate, per whole ventricle. All data were subjected to a two-way analysis of variance (ANOVA) to determine the effects of surgical procedure (banding...
A further least-squares-means analysis was performed to evaluate individual group differences. Statistical significance was defined as an overall alpha value of less than 0.05, with values adjusted by Bonferroni’s correction as appropriate for the number of groups. When they appear in a table, p values are not repeated in the text. Lack of a p value indicates that no statistically significant difference exists.

Results

Body Weight

All animals showed a normal growth pattern with incremental increases between groups killed at 9, 18, and 27 weeks (Table 1). At no time point was there a difference between banded and sham-operated animals in body weight. None of the animals displayed signs (e.g., edema, ascites, hepatic congestion) of congestive heart failure.

Hemodynamic Measurements

Because of the effects of anesthesia and surgery on catecholamine homeostasis, no hemodynamic measurements were attempted in animals designated for biochemical studies. Therefore, right ventricular pressures in 2 separate groups of 4 animals each was determined at 27 weeks after surgery to demonstrate that the initial loosely fitting band applied to the pulmonary artery eventually produced hemodynamically significant right ventricular pressure overload and to quantitate its magnitude. Significant elevations of right ventricular systolic pressures in banded over sham-operated animals (39 ± 3.7 vs. 20.8 ± 1.5 mm Hg, respectively; p < 0.01), were determined while end-diastolic pressures were similar (3.7 ± 2.3 vs. 1.3 ± 0.8 mm Hg, respectively; NS).

Ventricular Mass

Absolute right ventricular weight increased progressively and significantly in banded animals (Table 1). When ventricular mass was expressed as a fraction of body weight, a similar progressive increase from Group B9 to Group B18 was apparent; the slight further increase in Group B27 failed to reach statistical significance. However, the increase in relative right ventricular mass over corresponding sham-operated controls was progressive at all 3 time points and amounted to 62, 123, and 198%, respectively, at 9, 18, and 27 weeks (Figure 1). No changes in absolute or relative right ventricular mass occurred among groups of sham-operated animals. Thus, our method of loosely banding the pulmonary artery in young animals successfully produced a model of very slowly progressive, yet pronounced, right ventricular hypertrophy.

Left ventricular and septal mass, whether expressed in absolute or relative terms, remained unchanged in all experiment groups.

Tyrosine Hydroxylase Activity

Tyrosine hydroxylase activity per unit of tissue weight was decreased in the right ventricles of all 3 banded groups (Tables 2 and 3). However, when expressed as total enzyme activity per whole right ventricle, normal values were maintained in all banded groups.

Right atrial activity of tyrosine hydroxylase (expressed per unit tissue weight) rose progressively in banded and sham-operated animals throughout the experiment.

Table 1. Effects of Chronically Progressive Right Ventricular Pressure Overload on Body Weight and Absolute and Relative Ventricular Weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>RV mass (g)</th>
<th>RV body weight (mg/g)</th>
<th>LV plus S mass (mg/g)</th>
<th>RV/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B9</td>
<td>684 ± 18</td>
<td>0.63 ± 0.04*</td>
<td>0.92 ± 0.05*</td>
<td>1.19 ± 0.05</td>
<td>1.73 ± 0.05</td>
</tr>
<tr>
<td>B18</td>
<td>778 ± 14†</td>
<td>1.01 ± 0.08†</td>
<td>1.31 ± 0.11†</td>
<td>1.35 ± 0.05</td>
<td>1.73 ± 0.04</td>
</tr>
<tr>
<td>B27</td>
<td>890 ± 41†</td>
<td>1.23 ± 0.07†</td>
<td>1.41 ± 0.11†</td>
<td>1.48 ± 0.06</td>
<td>1.68 ± 0.08</td>
</tr>
<tr>
<td>S9</td>
<td>722 ± 33</td>
<td>0.40 ± 0.02</td>
<td>0.55 ± 0.01</td>
<td>1.30 ± 0.08</td>
<td>1.80 ± 0.10</td>
</tr>
<tr>
<td>S18</td>
<td>783 ± 51†</td>
<td>0.46 ± 0.03</td>
<td>0.59 ± 0.01</td>
<td>1.36 ± 0.06</td>
<td>1.73 ± 0.06</td>
</tr>
<tr>
<td>S27</td>
<td>928 ± 28†</td>
<td>0.45 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>1.51 ± 0.06</td>
<td>1.62 ± 0.04</td>
</tr>
</tbody>
</table>

RV, right ventricle; LV, left ventricle; S, interventricular septum; B9 or S9, banded or sham-operated animals 9 weeks after surgery. B18 or S18, banded or sham-operated animals 18 weeks after surgery; B27 or S27, banded or sham-operated animals 27 weeks after surgery; all results are expressed as mean ± SEM. *p < 0.01 vs. corresponding sham-operated group; †p < 0.01 vs. value listed in same column 1 line above.

Figure 1. Left panel: Absolute right ventricular weight in mg. Right panel: Right ventricular weight body weight ratio (relative right ventricular weight), in mg/g. Open bars, sham-operated animals at 9, 18, and 27 weeks after surgery (Groups S9, S18, and S27, respectively); hatched bars, animals at 9, 18, and 27 weeks after pulmonary artery banding (Groups B9, B18, and B27, respectively). Figures in the bottom of each bar, number of animals in each group.
Myocardial Norepinephrine concentrations were not proportional to the increase in right ventricular weight, so, as a net effect, a significant increase in myocardial norepinephrine concentrations was only noted for the comparison between all banded and sham-operated groups at 18 and 27 weeks.

Dopamine β-Hydroxylase Activities

Similar to the findings regarding tyrosine hydroxylase, dopamine β-hydroxylase activity per whole right ventricle remained without significant changes in banded animals throughout the experiment (Tables 1 and 2). Activity per unit weight of tissue was again lower in banded than in sham-operated animals. Right atrial activities of tyrosine hydroxylase (measured only at 9 and 18 weeks) tended to be slightly lower in banded than in sham-operated animals and showed no change over time.

Myocardial Norepinephrine

In all 3 groups of banded animals, right ventricular norepinephrine concentrations were significantly depressed (Tables 2 and 3). However, these reductions were not proportional to the increase in right ventricular weight, so, as a net effect, a significant increase in myocardial norepinephrine contents at 18 and 27 weeks in banded over sham-operated animals resulted (Figure 2). The progressive rise in norepinephrine content was statistically significant for the comparisons between all 3 banded groups as well as between banded and sham-operated groups at 18 and 27 weeks.

No appreciable difference was found in right atrial norepinephrine concentrations of all experiment groups.

### Table 2. Effects of Chronically Progressive Right Ventricular Hypertrophy on Indexes of Autonomic Innervation

<table>
<thead>
<tr>
<th>Group</th>
<th>TH (nmol/hr/g)</th>
<th>TH (nmol/hr/RV)</th>
<th>DBH (nmol/hr/g)</th>
<th>DBH (nmol/hr/RV)</th>
<th>NE (μg/g)</th>
<th>NE (μg/RV)</th>
<th>CAT (nmol/hr/g)</th>
<th>CAT (nmol/hr/RV)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>B9</td>
<td>38 ± 4 0*</td>
<td>23 ± 4 ± 3</td>
<td>265 ± 57*</td>
<td>161 ± 33</td>
<td>2 3 ± 0 15*</td>
<td>1 45 ± 0 05</td>
<td>32 2 ± 2 6*</td>
<td>20 5 ± 1 5</td>
<td>8</td>
</tr>
<tr>
<td>B18</td>
<td>34 ± 2 7*</td>
<td>34 ± 2 3 2</td>
<td>225 ± 42*</td>
<td>209 ± 22</td>
<td>2 5 ± 0 20*</td>
<td>2 45 ± 0 11*</td>
<td>32 8 ± 2 1*</td>
<td>32 6 ± 2 7*</td>
<td>8</td>
</tr>
<tr>
<td>B27</td>
<td>23 3 ± 2 0*</td>
<td>28 ± 1 2 2</td>
<td>130 ± 21*</td>
<td>153 ± 19</td>
<td>2 50 ± 13*</td>
<td>3 04 ± 0 19*</td>
<td>19 0 ± 1 4*</td>
<td>22 8 ± 1 4</td>
<td>9</td>
</tr>
<tr>
<td>S9</td>
<td>73 ± 6 5</td>
<td>29 ± 2 ± 3</td>
<td>500 ± 39</td>
<td>196 ± 13</td>
<td>4 05 ± 3 2</td>
<td>1 58 ± 1 0</td>
<td>46 9 ± 4 2</td>
<td>18 4 ± 1 7</td>
<td>6</td>
</tr>
<tr>
<td>S18</td>
<td>64 ± 10 3</td>
<td>29 ± 0 ± 4</td>
<td>554 ± 56</td>
<td>254 ± 38</td>
<td>3 96 ± 0 23</td>
<td>1 80 ± 0 13</td>
<td>40 0 ± 5 5</td>
<td>21 8 ± 1 3</td>
<td>6</td>
</tr>
<tr>
<td>S27</td>
<td>72 2 ± 6 6</td>
<td>32 2 ± 3 7</td>
<td>456 ± 29</td>
<td>205 ± 18</td>
<td>5 32 ± 0 37</td>
<td>2 40 ± 0 22*</td>
<td>40 2 ± 3 4</td>
<td>18 3 ± 2 3</td>
<td>6</td>
</tr>
</tbody>
</table>

*TH, tyrosine hydroxylase; DBH, dopamine beta-hydroxylase; NE, norepinephrine; CAT, choline acetyltransferase; RV, right ventricle. B9 or S9, banded or sham-operated animals 9 weeks after surgery; B18 or S18, banded or sham-operated animals 18 weeks after surgery; B27 or S27, banded or sham-operated animals 27 weeks after surgery. All results are expressed as mean ± SEM. †p < 0.01 vs corresponding sham-operated group. ‡p < 0.01 vs value listed in same column line above, *p < 0.01 vs all other groups.

Choline Acetyltransferase Activity

A marked increase in total right ventricular activity of choline acetyltransferase occurred in Group B18, while activities in the other 2 banded groups were not different from sham-operated controls (Table 2 and Figure 3). Choline acetyltransferase activity per gram of right ventricular tissue was lower in banded than in sham-operated animals.

### Discussion

Sympathetic and parasympathetic nerves play a major role in regulating cardiac function. Thus, great importance has been ascribed to the changes in markers of cardiac autonomic innervation occurring in cardiac hypertrophy and failure. Designed to complement earlier studies of acutely induced cardiac hypertrophy, the present investigation was undertaken to study whether the time course over which cardiac hypertrophy develops represents a variable influencing these changes. Our experiments yielded 2 major findings: 1) Our results demonstrate that, in contrast to acutely induced ventricular hypertrophy, parameters of sympathetic innervation are preserved or increased in slowly progressive cardiac hypertrophy. 2) In addition, indexes of parasympathetic innervation show transient elevations during the development of hypertrophy similar to those seen in acute ventricular hypertrophy.

The experiment design employed in the present study successfully produced a model of very slowly progressing cardiac hypertrophy.
advancing and, at the same time, pronounced right ventricular hypertrophy. The pronounced increases in right ventricular mass achieved by our method, 68, 123, and 198% over controls at 9, 18, and 27 weeks after banding, respectively, match and exceed those previously documented in acute experiments in this laboratory (52–125% increase) as well as those reported by others (usually 60–100% increase) in ventricular mass. Again, these findings may not be applicable to other models.

Furthermore, it is of interest that in several studies examining the catecholamine stores of failing human hearts, control tissues from hearts of patients with ischemic or valvular heart disease had normal or elevated norepinephrine concentrations. Although not documented, these patients presumably had long-standing cardiac hypertrophy. Naturally, these observations are difficult to interpret because, first, "normal" values are based on very small numbers of patients, and second, atrial rather than ventricular tissues were analyzed in most cases. Furthermore, there is evidence in the literature that at an early stage, even in acutely induced cardiac hypertrophy, normal ventricular norepinephrine stores may be maintained but only if the increase in relative cardiac weight is modest (25–42%). Our findings clearly demonstrate that even severe pressure overload and pronounced myocardial hypertrophy are not necessarily associated with neurotransmitter depletion and that the time course over which cardiac hypertrophy develops may represent the factor determining the fate of cardiac sympathetic nerves.

Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine biosynthesis. Decreased activities have been shown to parallel reductions in cardiac catecholamines and a causal relation has been postulated. It has been emphasized, however, that measured tyrosine hydroxylase activity does not predict catecholamine levels, probably because in vitro tyrosine hydroxylase assays are carried out in the presence of high cofactor and substrate concentrations. These conditions may obscure moderate changes in enzyme activity due to modification of tissue constituents or the enzyme molecule itself, one of the two mechanisms known to regulate tyrosine hydroxylase activity (the other being a change in tissue concentrations of enzyme protein).

The data obtained in the present study do not allow for pinpointing the mechanism for increased myocardial catecholamine stores. However, several observations may relate to the question of why our data are at variance with findings in models of acutely induced cardiac hypertrophy. The decrease in markers of symp-
pathetic innervation in the hypertrophied myocardium in models of acute pressure overload has been explained as sequelae of focal necrosis and subsequent fibrosis. Schmid and colleagues demonstrated early and sustained loss of tyrosine hydroxylase activity in the ischemic canine heart, and Borchard has shown histochemically that the attrition of sympathetic nerve fibers was disproportionate to the degree of cardiac hypertrophy. In human atria, the depletion of norepinephrine and tyrosine hydroxylase activity correlates directly with the degree of cardiac hypertrophy. Focal areas of fibrosis have been documented in experiment animals after abruptly raising right or left ventricular afterload, while animals with a much greater degree of cardiac hypertrophy due to congenital heart disease showed no myocardial scarring. Experimental imposition of slowly rising pressure overload, then, would not be expected to cause focal necrosis or loss of nerve fibers, which, indeed, corresponds well with our findings. The net gain in right ventricular norepinephrine may be due to an increase in the amount of norepinephrine stored per nerve terminal or to an increase in the number of sympathetic terminals. The young age of our experiment animals raises the possibility that the plasticity of autonomic innervation present early postpartum or that a growth-related neuron-target organ relation may have been preserved to a greater degree than in adult animals, resulting in sprouting of sympathetic nerves. However, the relative maturity of guinea pigs at birth, the rapid postnatal maturation of cardiac nerves in small animals and the lack of regenerative capacity in adult animals make an increase in neurotransmitter per nerve ending more likely. The slight increase in right ventricular catecholamines noted in sham-operated animals in the absence of pressure stress corresponds with similar observations in other species.

In the absence of turnover data, the physiologic meaning of increased myocardial catecholamines must essentially remain speculative. However, evidence from the literature suggests that augmentation of contractile function or even maintenance of baseline performance in the compensated hypertrophied heart may depend on intact cardiac norepinephrine stores. Thus, the transition into the decompensated stage of congestive failure with its associated marked depletion of cardiac catecholamines commonly has been linked to an "exhaustion" of inotropic adrenergic support. Viewed in this context, maintenance of normal or elevated ventricular norepinephrine content may relate to the fact that, in spite of severe pressure overload and massive ventricular hypertrophy, our experiment animals did not develop detectable signs of heart failure. Similarly, our results may be of relevance to previous observations of normal myocardial mechanics in models of slowly developing cardiac hypertrophy induced by volume overload or loose pulmonary artery banding, which contrast with the discrete abnormalities in cardiac function seen in acutely induced hypertrophy.

Choline acetyltransferase activity provides an index of parasympathetic innervation; its fate in ventricular hypertrophy markedly differing from that of sympathetic markers. Very similar to our findings of a temporary increase in the enzyme's activity, Lund et al demonstrated increased activity followed by a return to normal in guinea pigs with acute pulmonary banding; when a less constricting band was used, the rise in activity occurred later. They concluded that an increase in choline acetyltransferase activity occurs just before the peak increase in heart weight, a statement also appropriate to our results. The meaning of these changes is unknown, and they may well represent only epiphenomena. However, based on the recognized antagonism between sympathetic and parasympathetic influences, a pathogenetic role of withdrawal of parasympathetic tone has been suggested for cardiac decompensation in the cardiomyopathic hamster. Conversely, an increase in parasympathetic function at a critical time point may be regarded as a "brake" retarding the progression of cardiac hypertrophy either by interfering with the postulated trophic role of sympathetic amines or by inhibiting directly the positive inotropic state said to promote cardiac growth. The physiologic substrate for increases in cardiac choline acetyltransferase may be, similar to the sympathetic nervous system, an increase in enzyme per nerve terminal or an increase in the number of terminals. However, in contrast to adrenergic nerves, regenerative sprouting of nerve fibers after partial parasympathetic denervation has been documented and could represent the mechanism involved.

Our study has certain limitations. No data on the performance of the hypertrophied ventricles was obtained, and only three points in time were examined. An initial decrease in cardiac catecholamines similar to the observations of Spann et al cannot be ruled out, and neither can it be certain that such a drop would not have occurred had we waited for congestive heart failure to develop. In addition, correlation of physiologic and biochemical findings with histologic and biochemical techniques as well as corroboration of our findings with those in different species would be desirable. Future work will be directed at answering these and similar questions.

The cardinal finding of our study is the demonstration that severe ventricular hypertrophy induced by slowly progressive pressure overload is not associated with depression of sympathetic indexes but with maintenance of normal enzyme activities and increases in cardiac norepinephrine stores. Our results strongly support the concept that the time course over which pressure overload is imposed is a major factor determining the fate of cardiac sympathetic innervation and, thus, an important modulator of the nature of cardiac hypertrophy. Conversely, choline acetyltransferase activity showed a transient rise quite similar to findings in acute pressure overload. These results indicate that autonomic cardiac nerves may play differential roles in acute and chronic ventricular hypertrophy. Having been obtained in a physiologically relevant model of cardiac hypertrophy, our findings may have
important clinical significance in furthering our understanding of this condition in clinical medicine.

Acknowledgments
We wish to thank Ms. Carol Whites and Mr. Alberto Subieta for their expert technical assistance.

References
17. Sass J. Mechanism of myocardial catecholamine depletion in cardiac hypertrophy and failure in rabbits Jpn Circ J 1970;34:391–403

Lindpaintner I, Autonomic Innervation in Chronic Cardiac Hypertrophy 61


Boerth RC. Postnatal development of myocardial adrenergic mechanisms in the cat (abstract). Circulation 1973 (suppl IV to vols 7, 8), 36.


Key Words: right ventricular hypertrophy, cardiac hypertrophy, sympathetic nervous system, parasympathetic nervous system, norepinephrine, tyrosine hydroxylase, dopamine β-hydroxylase, choline acetyltransferase.
Effects of chronic progressive myocardial hypertrophy on indexes of cardiac autonomic innervation.
K Lindpaintner, D D Lund and P G Schmid

Circ Res. 1987;61:55-62
doi: 10.1161/01.RES.61.1.55

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/61/1/55