Norepinephrine Turnover in the Heart and Spleen of the Cardiomyopathic Syrian Hamster

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ABSTRACT

Although a reduction in myocardial norepinephrine stores in cardiac hypertrophy and congestive failure is well documented, norepinephrine turnover has been inadequately studied in such hearts. We compared norepinephrine turnover in control and cardiomyopathic hamsters by following the decline in specific activity of myocardial norepinephrine after labeling with an intraperitoneal tracer dose of $^3$H-norepinephrine. Adult myocardial norepinephrine concentrations were not attained until 4 weeks of age in both strains. There was no difference in the rate constant ($K$) for myocardial norepinephrine turnover ($0.107 \pm 0.004$ hours$^{-1}$ vs. $0.100 \pm 0.005$ hours$^{-1}$) in the two strains of hamsters during the neonatal period. In young control hamsters, $K$ fell to $0.064 \pm 0.004$ hours$^{-1}$, but that for age-matched hamsters with mild cardiac hypertrophy was $0.102 \pm 0.001$ hours$^{-1}$ ($P < 0.001$). There was little change in $K$ as control hamsters aged. With the development of more severe hypertrophy in cardiomyopathic hamsters, cardiac norepinephrine decreased and resting $K$ rapidly increased to approach the value obtained when hamsters were subjected to immobilization stress ($0.302 \pm 0.013$ hours$^{-1}$). The maximum achievable $K$ remained the same for both control and dystrophic hamsters even during terminal disease. Prolonged immobilization led to a reduction in cardiac norepinephrine in both strains. Ganglionic blockade of failing hamsters completely restored the levels of both cardiac norepinephrine and $K$ to control values. Splenic noradrenergic nerves showed no change in $K$, norepinephrine content, or maximum $K$ during cardiac decompensation. We conclude that, in the late stages of hamster cardiomyopathy, there is a progressive and possibly specific increase in cardiac sympathetic tone which leads to a concomitant decrease in cardiac norepinephrine. With the loss of sympathetic reserve, congestive failure supervenes.

The normal heart is richly supplied with noradrenergic sympathetic nerves. This innervation contributes little to intrinsic myocardial function (1) but is a most important mechanism for the elevation of cardiac output in response to a physiological stress such as exercise (2). In the absence of a catecholamine stimulus, cardiac output can be increased solely by the Frank-Starling mechanism (3). Dilated or noncompliant hearts, however, cannot take further advantage of the length-tension relationship and thus depend on sympathetic support to maintain cardiac output (4).

There is considerable evidence for sympathetic dysfunction in the hearts of humans with congestive heart failure and animals with experimentally induced cardiac disease. Several investigators have demonstrated a decrease in the norepinephrine concentration (5–8) and the norepinephrine storage capacity (5) of enlarged hearts. Covell et al. (9) have shown that the failing heart does not respond adequately to stimulation of its sympathetic nerves. Diminished levels of tyrosine hydroxylase, felt to be the rate-limiting enzyme for norepinephrine biosynthesis, have also been found in such hearts (7, 10).

In contrast to the abundant measurements of norepinephrine levels in the failing and hypertrophied heart, very little information is available concerning its turnover and synthesis rates in such hearts. Spann et al. (11) have demonstrated that the cardiac norepinephrine turnover time is identical in normal guinea pigs and guinea pigs with heart failure induced by supravalvular aortic constriction. These guinea pigs were models of acute heart failure, since the aortic band was placed only 10 days prior to assay; hence, their autonomic function might not be similar to that associated...
with the chronic form of cardiac hypertrophy secondary to human heart disease. Fischer et al. (12), in experiments with rat hearts hypertrophied secondary to banding of the abdominal aorta, found no change in norepinephrine turnover time in mildly enlarged hearts and somewhat of an increase in moderately enlarged ones. This model of aortic coarctation, although classic in biochemical studies of pressure-overload hypertrophy, exhibits a marked rostral-caudal difference in blood pressure which in turn may contribute significantly to the observed cardiac sympathetic response.

Angelakos et al. (8) have performed preliminary studies on norepinephrine metabolism in the hearts of cardiomyopathic hamsters, but they measured labeled cardiac norepinephrine at only a single section of turnover rates.

Surgical models of heart failure requiring thoracotomy and vascular banding can exhibit artifactual changes in sympathetic nervous function secondary to the operative procedure rather than the myocardial dysfunction. The cardiomyopathic hamster provides a natural model of chronic congestive heart disease (13). We therefore decided to examine norepinephrine turnover in the cardiac and splenic sympathetic nerves of these animals and to relate our findings to the course of their heart disease.

Methods

Cardiomyopathic (Bio 14.6) and control (Bio 2.4) Syrian hamsters of both sexes 8-340 days of age were used in these experiments. The hamsters were age and sex matched. All hamsters were allowed at least 2 weeks to acclimatize to our laboratory animal facility following delivery from the breeder (Bio Research Inst., Cambridge, Massachusetts). Hamsters were fed a standard laboratory diet. A 12-hour light, 12-hour dark cycle was maintained in the animal housing area.

Each hamster was given 4 µc/100 g of 7-3H-dl-norepinephrine (3H-NE) (New England Nuclear Corp., 7.2 c/mmole) in 150 µlitters of 10-4 N acetic acid by intraperitoneal injection. Preliminary studies demonstrated that less than 0.5% of the radioactivity was detectable in the peritoneal cavity 20 minutes after the injection.

The rate of change of the specific activity of norepinephrine was measured between 2 and 26 hours after labeling. At appropriate times after the injection, the hamsters were killed by decapitation, and their hearts and spleens were removed and rinsed. The ventricles were dissected free of the atra and great vessels, and the organs were then immediately frozen on Dry Ice. The tissues were weighed and then homogenized in 6 volumes of iced 0.4N perchloric acid containing 0.4% ethylenediaminetetraacetic acid (EDTA) and 3 mg/100 ml of sodium metabisulfite. The homogenate was centrifuged at 30,000 g for 30 minutes. Catecholamines were absorbed from the supernatant fluid onto an alumina column at pH 8.6 and eluted with 4 ml of 0.2 N acetic acid. A 500 µliter sample of the alumina eluate was added to 13 ml of Bray’s solution and counted in a Packard liquid scintillation counter. Counting efficiency was calculated by the subsequent addition of 3H-toluene as an internal standard. Norepinephrine was measured in the alumina eluate by conversion to its trihydroxyindole derivative by the modification of O’Hanlon et al. (14) of the method of Laverty and Taylor. Fluorescence was read in an Amino-Bowmann spectrophotofluorometer. Norepinephrine recovery varied between 86% and 94%.

Under steady-state conditions, the rates of synthesis and removal of norepinephrine are equal. Thus, the overall rate of norepinephrine synthesis can be expressed as K[NE] (15), where K is the rate of change of the specific activity of the labeled norepinephrine lost per unit time (the turnover rate constant) and [NE] is the steady-state level of norepinephrine. K[NE] is the norepinephrine turnover rate. The turnover rate constant was calculated from the rate of decline of the logarithm of the specific activity of the labeled norepinephrine stores (regression coefficient) (15). Analysis of variance was used for calculating the standard error of the regression coefficient, the significance of the regression coefficients, and the difference between them. A minimum of 12 hamsters (from at least six litters) in each of the control and cardiomyopathic groups was used for each turnover study.

Initial studies using 30-45 hamsters of each strain and 3-7 time points showed that first-order kinetics were obeyed for both the heart and the spleen over the time period examined (2-26 hours after injection). Since altered circulatory dynamics in congestive heart failure could affect the uptake of the administered norepinephrine, we did not consider a comparison of the absolute uptake of tracer to be informative.

The Syrian hamster hibernates; thus, we were unable to use cold as a cardiovascular stress. Therefore, the hamsters were stressed by taped immobilization to a board. Each stress experiment consisted of three groups of (six or more) hamsters for each of the strains. All of the hamsters were given 4 µc/100 g 3H-NE intraperitoneally as described earlier. Two hours after the injection, one group of hamsters was killed, a second group was immobilized, and a third was left undisturbed. Eight hours after the injection, the second and third groups were killed. Norepinephrine content and turnover were calculated as described earlier. The first group served as an initial point for both turnover studies.

Chlorisondamine (Ecolid chloride), a ganglionic blocker which does not enter the central nervous system, was used to inhibit peripheral sympathetic activity in some of our studies; 10 mg/kg was given by intraperitoneal injection every 6 hours for 24 hours to the treated hamsters. Control hamsters received the distilled water vehicle.

Hamsters were alternated in a set and timed order between groups or strains or both at every stage of these experiments. Results are expressed as means ± SE.

1Chlorisondamine was generously provided by the CIBA Pharmaceutical Co., Summit, N. J.
Results

The disease course of our cardiomyopathic hamsters can be divided into several stages as previously reported (13). During the first or prenecrotic stage, the hamsters show no clinical or histological evidence of disease. Between 40 and 70 days of age (necrotic stage), areas of focal myolysis and cellular infiltrates can be seen throughout the myocardium. Healing occurs over the subsequent 40 days. The heart hypertrophies and dilates gradually over the next 250 days. Little, if any, active myolytic disease is in evidence at this time. Hamsters with cardiac enlargement were classified as having early hypertrophy (150-180 days), moderate hypertrophy (250-300 days), severe hypertrophy (older than 300 days), and congestive failure (older than 300 days with hepatic congestion, ascites, subcutaneous edema, and at times pulmonary edema).

BODY AND VENTRICULAR WEIGHTS

Body weight did not differ significantly between the two groups of hamsters except between failing hamsters (older than 300 days) and their age-matched controls. The former had body weights of 160.3 ± 13.7 g and the latter 108.4 ± 2.7 g (P < 0.01). Hamsters with failure were separated from those of the same age with only hypertrophy on the basis of body weight and appearance at autopsy (the selection was made prior to any biochemical measurements). Ventricular wet weights are illustrated in Figure 1. The differences became significant at the stage of early hypertrophy (150-180 days). Ventricular weights of cardiomyopathic hamsters 300-340 days old with hypertrophy or hypertrophy and failure did not differ significantly.

CARDIAC NOREPINEPHRINE CONCENTRATION AND CONTENT

Cardiac norepinephrine concentration (Fig. 2) and content (Fig. 3) were measured at each stage of the disease process. In both control and diseased hamsters, cardiac norepinephrine concentration did not reach adult levels until approximately 1 month following birth. There was no significant difference in norepinephrine concentration between the two groups at 8 days of age. The cardiomyopathic hamsters had a slightly but significantly greater concentration of cardiac norepinephrine than did the control hamsters by 2.5 weeks of age. This increase persisted through the stage of moderate hypertrophy. During the stage of late hypertrophy and failure, the concentration of ventricular norepinephrine decreased to approximately 37% of that of matched controls and to 31% of the norepinephrine concentration found prior to the onset of hypertrophy. The decrease in norepinephrine concentration in hamsters 300-340 days old with hypertrophy with or without failure was significant compared with that of cardiomyopathic hamsters at earlier stages of the disease.

Norepinephrine content per heart (right and left ventricle) does not reflect dilution of sympathetic nerve endings by the hypertrophied myocardium. Using this mode of expressing our data, basically the same pattern of norepinephrine stores was seen.

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At the age of 45–65 days, there appeared to be a small but significant relative increase in total ventricular norepinephrine stores in the cardiomyopathic hamsters. With the progression of hypertrophy, norepinephrine stores eventually decreased. The cardiac norepinephrine content at 300–340 days in hamsters with hypertrophy with or without failure was significantly less than that in hamsters during the earlier stages of the disease. Norepinephrine content in hamsters with failure was approximately 64% of that in matched controls and approximately 53% of the peak norepinephrine content found in younger cardiomyopathic hamsters.

VENTRICULAR NOREPIINEPHRINE TURNOVER

The results of the norepinephrine turnover studies in ventricles of resting hamsters are summarized in Table 1. As can be seen from the study illustrated in Figure 4, the decline of specific activity followed first-order kinetics over the time measured. The cardiac norepinephrine turnover rate constant was identical for both groups of hamsters in the neonatal period. This rate decreased with the attainment of adult levels of ventricular norepinephrine in normal hamsters but remained fixed at neonatal levels in cardiomyopathic ones. With the onset of the late stages of the cardiomyopathy, the cardiac norepinephrine turnover rate constant increased greatly.

The norepinephrine turnover rate per gram of ventricle was identical in both groups of very young hamsters. At the necrotic stage and prior to the onset of cardiac hypertrophy, there was a 70% increase in the turnover per gram of myopathic heart compared with the level in control hearts (Table 1). This increase persisted until the stage of failure when the high rate constant was offset by the decrease in norepinephrine concentration and turnover fell sharply.

When norepinephrine turnover per total ventricular mass was examined (Table 1), we found a persistent 75–90% increase in the turnover rate of cardiomyopathic hamsters compared with that of controls beginning at 45–65 days of age.

STRESS TURNOVER

To determine whether the maximum achievable rate constant was affected by the disease process,
hamsters were stressed as described in Methods. Cardiomyopathic hamsters and matched controls were examined at each stage of life from early hypertrophy to failure. Neither age nor stage of the disease had any bearing on the maximum achievable rate constant for cardiac norepinephrine turnover. This maximum rate constant was identical for both cardiomyopathic and control hamsters (0.302 ± 0.013).

We did not find significant differences in norepinephrine stores between resting hamsters and those subjected to 6 hours of immobilization stress, but a gradual decline may not be manifested during such a brief period. Thus, to determine whether a chronic elevation in turnover rate could result in a reduction of cardiac norepinephrine, we immobilized a group of hamsters for 24 hours. We observed (Table 2) a 40% decrease in cardiac norepinephrine in the stressed control hamsters and a 47% decrease in those with the cardiomyopathy.

CARDIAC RESERVE FOR NOREPINEPHRINE TURNOVER

We examined cardiac sympathetic reserve by comparing resting and stressed turnover rates. Figure 5 illustrates the norepinephrine turnover

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>NE/heart (ng)</th>
<th>NE/g heart (ng/g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Stress</td>
</tr>
<tr>
<td>Control</td>
<td>488.5 ± 43.3</td>
<td>293.1 ± 40.2*</td>
</tr>
<tr>
<td>Myopathic</td>
<td>322.0 ± 51.8</td>
<td>171.6 ± 29.1*</td>
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All values are means ± SE.

*P < 0.02.
†P < 0.05.
rate per heart per hour in both resting and stressed states. The resting turnover rate was 21-30% of that during stress in control hamsters at all ages. Cardiomyopathic hamsters with early and moderate hypertrophy had resting turnover rates 33-34% of those achievable with stress. Hamsters with severe hypertrophy had resting rates 68% of those obtained under stress, and hamsters with failure had resting rates 78% of those obtained from their stressed colleagues. The identical turnover reserve data apply, of course, not only for turnover rate per hour but also for the rate constant alone or for turnover rate per gram of heart.

GANGLIONIC BLOCKADE

We wished to determine whether our observed high turnover rates were indeed indicative of an actual increase in sympathetic tone. Therefore, we examined cardiac norepinephrine turnover following peripheral ganglionic blockade with chlorisondamine (Table 3). After ganglionic blockade, both failing and control hearts exhibited identically low rate constants for norepinephrine turnover. In addition, there was a complete restoration of cardiac catecholamine stores in the failing hamsters.

SPLENIC NOREPINEPHRINE TURNOVER

In all hamsters but sucklings, splenic norepinephrine turnover was also studied (Table 4). We found a great deal of variability in splenic weight and norepinephrine content as well as considerable scatter in calculated specific activity. We could not definitely account for the great differences in splenic weights, but we believe it was due to variations in blood content. As we recorded our findings per total spleen rather than per gram of organ, our findings were not affected by this variability in organ weight. The rate constant for splenic norepinephrine turnover appeared to be consistently higher in cardiomyopathic hamsters throughout life and bore no relationship to the disease state. The maximum achievable rate constant for splenic norepinephrine turnover was the same for both control and failing hamsters. In contrast to data obtained for the heart, no loss of turnover reserve was seen in severely diseased hamsters.

Discussion

The present observations characterize norepinephrine turnover in hearts of control and cardiomyopathic hamsters from infancy to old age and during resting and stress states. Our turnover data should not be accepted as a literal numerical measure of norepinephrine synthesis rate or sympathetic tone. The experimental rate constant is a weighed mean of the rate constants of possibly several norepinephrine pools—some of these pools may bear no relationship to actual sympathetic activity (15). Norepinephrine turnover under steady-state conditions can provide, however, a valid comparative assessment of the norepinephrine synthesis rate (15). The rate constant itself is independent of catecholamine content and, providing it can be shown to vary appropriately with sympathetic tone, can be thought of as a functional index of mean neuronal activity.

Cardiac norepinephrine stores required approximately 4 weeks following birth to attain adult concentrations in both groups of hamsters. A similar delay in the maturation of sympathetic innervation has been found in other mammalian hearts (16, 17). Our infant control hamsters had

<table>
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<th>TABLE 3</th>
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<tr>
<td><strong>Effect of Chlorisondamine on Cardiac Norepinephrine Metabolism</strong></td>
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<tr>
<td>NE/heart (ng)</td>
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<tr>
<td>NE/g heart (ng/g)</td>
</tr>
<tr>
<td>Rate constant (K) (hours⁻¹)</td>
</tr>
<tr>
<td>Half-life (hours)</td>
</tr>
</tbody>
</table>

All values are means ± SE.

* P < 0.001 for cardiomyopathic untreated vs. control untreated.

† P < 0.001 for cardiomyopathic treated vs. cardiomyopathic untreated.

‡ P < 0.05 for control treated vs. control untreated.
higher cardiac norepinephrine turnover rate constants than did their adult cohorts. This difference could be an artifact secondary to the handling of suckling hamsters. However, infant hamsters were injected with extreme care and neither they nor their mothers appeared to be disturbed by the brief injection process. Whether a similar decrease in the turnover rate constant accompanies the maturation of cardiac norepinephrine stores in other species has not been determined.

In contrast to our findings in control hamsters, no fall in the relatively high turnover rate constant of infancy accompanied the maturation of hamsters destined to develop the cardiomyopathy. Our observation that the ventricular norepinephrine stores of young cardiomyopathic hamsters were increased relative to those of controls supports similar findings by Angelakos et al. (8). This increase of both stores and rate constant led to a fivefold in response to a cardiac stress. During the late stages of the dystrophy, however, norepinephrine turnover rate in the resting ventricle rapidly approached the maximum achievable under stress, leaving failing hamsters with little, if any, reserve. This high basal turnover rate constant did not compensate for a concomitant reduction in the steady-state concentration of norepinephrine; thus, a fall in norepinephrine turnover per gram of heart accompanied the onset of congestive failure.

Angelakos et al. (8) have examined the noradrenergic nerve endings of the dystrophic hamster heart by the formaldehyde fluorescence histochemical technique of Falk and Hillarp. There was an increase in the fluorescence intensity of the nerve terminals of the cardiomyopathic hearts compared with that of the controls in all but the final stages of the disease. Distinct focal areas, perhaps regions of necrosis, were observed where no catecholaminergic fibers were present. There is a distinct possibility, then, that our observations were really a manifestation of catecholamine loss secondary to a degenerative process at the nerve terminal rather than the result of an increase in sympathetic tone. With immobilization stress, we could maintain an

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**TABLE 4**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Spleen weight (mg)</th>
<th>NE/spleen (ng)</th>
<th>Rate constant (hours⁻¹)</th>
<th>Half-life (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-65</td>
<td>114.0 ± 3.6</td>
<td>202.1 ± 7.6*</td>
<td>105.4 ± 4.3</td>
<td>0.075 ± 0.012</td>
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<tr>
<td>150-180</td>
<td>113.4 ± 9.8</td>
<td>258.7 ± 23.4*</td>
<td>124.9 ± 8.1</td>
<td>0.087 ± 0.022</td>
</tr>
<tr>
<td>250-300</td>
<td>213.0 ± 20.0</td>
<td>194.8 ± 12.0</td>
<td>81.2 ± 9.3</td>
<td>0.062 ± 0.017</td>
</tr>
<tr>
<td>300-340</td>
<td>203.0 ± 54.8</td>
<td>189.8 ± 39.8</td>
<td>134.7 ± 24.6</td>
<td>0.050 ± 0.100</td>
</tr>
<tr>
<td>329-340</td>
<td>83.5 ± 3.6</td>
<td>241.4 ± 44.6†</td>
<td>84.6 ± 4.6</td>
<td>0.055 ± 0.057</td>
</tr>
<tr>
<td>300-340 +</td>
<td>66.9 ± 4.0</td>
<td>128.5 ± 16.2*</td>
<td>72.6 ± 4.2</td>
<td>0.263 ± 0.126</td>
</tr>
<tr>
<td>hypertrophy</td>
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<td>and failure</td>
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All values are means ± SE. Half-life = 0.693/K.

* Differs from control at P < 0.001.
† Differs from control at P < 0.01.
‡ Hamsters were stressed by immobilization for 6 hours.

Under steady-state conditions may be a reflection of the maximum cardiac sympathetic tone. We found no impairment of the maximum turnover rate constant even during terminal disease. Our control hamsters and those with early heart disease could increase cardiac norepinephrine turnover three- to fivefold in response to a cardiac stress. With immobilization stress, we could maintain an
elevated cardiac norepinephrine turnover rate constant similar to that found in failing hamsters in our control hamsters. Under these conditions, the steady-state content of cardiac norepinephrine fell dramatically, closely simulating the picture of the steady-state content of cardiac norepinephrine fell similar to that found in failing hamsters in control values.

Cardiac decompensation did not affect the rate constant, norepinephrine stores, or sympathetic reserve of the spleen.

We conclude, therefore, that there is a progressive and possibly specific increase in cardiac sympathetic tone in the later stages of hamster cardiomyopathy. With a worsening of the disease state, the input-output relationships of the post-ganglionic cardiac sympathetic nerves approach those achieved with severe stress. Eventually, norepinephrine biosynthesis can no longer compensate for the mounting demand, and steady-state levels of norepinephrine fall. Ultimately, cardiac norepinephrine turnover per unit of myocardial mass declines. With the loss of sympathetic reserve, congestive failure supervenes.

Acknowledgment

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