Changes in parasympathetic and sympathetic neurochemical indices in hearts of myopathic hamsters

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Abstract

Sympathetic neurochemical indices in heart are increased in Syrian golden hamsters with skeletal and cardiac myopathy. The possibility that parasympathetic neurochemical indices might be altered was investigated in myopathic and normal hearts by measuring activity of choline acetyltransferase involved in acetylcholine synthesis. Confirming a previous report, tyrosine hydroxylase activity increased in failing myopathic hearts and norepinephrine concentration decreased. Extending previous work, tyrosine hydroxylase and dopamine-beta-hydroxylase activities in myopathic hearts demonstrated progressive, age-related increases. These changes were associated with reduced choline acetyltransferase activity in hearts of older myopathic hamsters (180 to 300-plus days). Decreases tended to be more pronounced in hamsters with cardiac hypertrophy and fluid retention (290–360 days old). Neurochemical evidence of increased sympathetic indices (dopamine-beta-hydroxylase activity) was detected at 30 days of age. Evidence of decreased parasympathetic indices (choline acetyltransferase activity) was detected at 180 days of age and persisted through terminal phases of heart failure. This study demonstrated that there are abnormalities in cardiac parasympathetic as well as cardiac sympathetic indices in myopathic hamsters.
Introduction

Choline acetyltransferase, catalyzing acetylcholine synthesis, and tyrosine hydroxylase, catalyzing the rate-limiting step in norepinephrine synthesis, may be affected differently in experimental heart disease. For example, in the pressure-overloaded heart, choline acetyltransferase activity tends to be maintained [20,22], whereas tyrosine hydroxylase activity is reduced [22]. In other experimental models of heart diseases, however, relative changes in parasympathetic and sympathetic neurochemical indices have not yet been investigated.

Sympathetic neural indices in heart are increased in Syrian golden hamsters with skeletal myopathy. Sole and co-workers, for example, found markedly increased norepinephrine turnover and tyrosine hydroxylase activity (24–26). The increased sympathetic indices associated with cardiomyopathy appeared to differ from the reduced indices associated with pressure overload. In the present study, we investigated parasympathetic as well as sympathetic indices in hearts of Syrian golden hamsters with skeletal myopathy. Our goals were to learn whether choline acetyltransferase activity becomes altered in myopathic hearts, at what age, and in what relationship to sympathetic neural changes.

Materials and Methods

Male and female Syrian golden hamsters (Bio 2.4) and their counterparts with hereditary skeletal myopathy (Bio 14.6) (Bio Research Inst., Cambridge, MA) were obtained at age 20 days and maintained 3 to a cage in the University of Iowa Animal Facility until sacrifice and study. The two groups were treated similarly and given a standard laboratory diet (Tecklab, Winfield, IA) and water ad libitum.

After sacrifice by cervical spinal dislocation, the hearts were dissected rapidly over ice, weighed, and stored in liquid nitrogen.

Biochemical determinations

Norepinephrine and dopamine were assayed in a micro-modification of the radioenzymatic method of Coyle and Henry [10,23]. The frozen tissue samples were homogenized with a Tekmar Tissumizer (Cincinnati, OH) in 50 vols. of cold 0.1 N perchloric acid and centrifuged at 4°C at 12,600 × g in an Eppendorf microfuge for 4 min. Dopamine and norepinephrine in the supernatant were converted to O-methyl analogues in the presence of catechol-O-methyl transferase and S-adenosylmethionine ([3H]methyl) (spec. act. 11.6 Ci/mm mol; New England Nuclear). Labeled normetanephrine and 3-methoxytyramine were extracted and the former was converted to vanillin ([3H]methyl) by metaperiodate cleavage. Methoxytyramine ([3H]methyl) was separated by solvent extraction and counted in a liquid scintillation counter.

Both ventricles were homogenized (20 vols. of ice-cold 5 mM potassium phosphate, 0.1 mM EDTA (pH 7.4) per g wet weight tissue) using four 10-s bursts of the Tekmar Tissumizer at a setting of 70. Ten per cent Triton X-100, a non-ionic
detergent, was added to the homogenate to give a concentration of 0.2% (v/v). Choline acetyltransferase activity was measured as previously described using a 15-min incubation at 37°C [16,17,19,20]. Tyrosine hydroxylase activity was measured by the procedure of Coyle using a 10-min incubation at 37°C [8,23]. The final concentrations of tyrosine and 2-amino-4-hydroxy-6, 7-dimethyl-tetrahydropteridine were 0.2 mM and 1.0 mM, respectively. Dopamine-β-hydroxylase was measured using a 20-min incubation at 37°C by the procedure of Coyle and Axelrod [9,23]. Duplicate protein determinations were performed according to the method of Lowry et al. [15].

A two-factor analysis of variance was used to analyze differences between control and myopathic groups. Multiple comparisons among group means were accomplished using the multiple-t method with an overall level of significance of $P < 0.05$ [3].

Results

Five different age groups of myopathic (Bio 14.6) and age-matched control (Bio 2.4) hamsters were studied. Younger hamsters from either source (30, 90 and 180 days old) had age-related increases in heart weight but no differences between control and myopathic groups were detected (Table I). Older myopathic hamsters (290–360 days old) had significantly greater ($P < 0.05$) heart weights than control hamsters and 6 myopathic hamsters had evidence of heart failure: ascites, hepatic congestion, and peripheral edema.

Indices of sympathetic innervation

Tyrosine hydroxylase (TH) activity increased progressively with age in the stellate ganglia of myopathic hamsters but not in control hamsters (Fig. 1). Increased TH

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Heart weight (mg)</th>
<th>Body weight (g)</th>
<th>Heart weight/body weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Myopathic</td>
<td>Control</td>
</tr>
<tr>
<td>30 (6)</td>
<td>185 ± 10</td>
<td>172 ± 6</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>90 (6)</td>
<td>325 ± 17</td>
<td>285 ± 12</td>
<td>111 ± 4</td>
</tr>
<tr>
<td>180 (6)</td>
<td>388 ± 9</td>
<td>387 ± 23</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>290–360 (9)</td>
<td>415 ± 12</td>
<td>515 ± 17 *</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>(hypertrophy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>290–360 (6)</td>
<td>436 ± 15</td>
<td>545 ± 17 *</td>
<td>133 ± 4</td>
</tr>
<tr>
<td>(failure)</td>
<td></td>
<td></td>
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</tbody>
</table>

* Differs from control, $P < 0.05$. 

TABLE I

HEART WEIGHT, BODY WEIGHT AND HEART WEIGHT RATIOS OF CONTROL AND CARDIOMYOPATHIC HAMSTERS

All values represent means ± S.E.M. Numbers in parentheses denote the number in each group.
Fig. 1. Tyrosine hydroxylase activity (nmol, mg prot $^{-1}$, h $^{-1}$) in the superior cervical ganglia, stellate ganglia, and adrenals in control and cardiomyopathic hamsters at various ages. Hypertrophy and failure group were 290–360 days old. Each value is mean $\pm$ S.E.M. for at least 6 animals. *, significantly different from control ($P<0.05$).

activity also occurred in the adrenal gland of myopathic hamsters compared to control hamsters, but not in the superior cervical ganglia. In parallel with the TH activity in stellate ganglia, TH activity and dopamine-$\beta$-hydroxylase (DBH) activity increased in myopathic hearts to greater levels than in control hearts (Fig. 3). When values were evaluated per unit weight, norepinephrine, but not dopamine concentration was markedly reduced in failing myopathic hearts (Fig. 2) and tyrosine hydroxylase and dopamine-$\beta$-hydroxylase activities were maintained. In terms of age-related changes, increases in DBH activity in myopathic hearts were evident at all ages, whereas, increases in TH activity were evident at 180 days and older (Fig. 3). There was no appreciable difference between old myopathic hamsters with heart failure and those with myocardial hypertrophy alone with respect to these changes.

Indices of parasympathetic innervation

In time course of change in choline acetyltransferase (CAT) activity in myopathic hearts differed markedly from that of TH activity (Fig. 3). Whereas TH activity increased progressively from 30 to 360 days of age in myopathic hearts, total CAT activity (Fig. 3) increased early (from 30 to 90 days of age) in parallel with CAT activity in control hearts and then decreased progressively in the myopathic heart so
that heart failure was associated with markedly reduced CAT activity compared to age-matched control hamsters' hearts. Decrease in CAT activity tended to be most pronounced in failing myopathic hearts.

Discussion

Syrian golden hamsters with cardiomyopathy and heart failure had late reductions in choline acetyltransferase activity, an index of parasympathetic innervation. The parasympathetic system was affected differently than the sympathetic system.

In the myopathic heart, sympathetic neurochemical indices increased progressively to the stage of heart failure. Increases were detected in stellate and adjacent ganglia, which are the origin of sympathetic innervation of heart. Changes were not detected in superior cervical ganglia. Increases also were noted in the adrenal and these became marked in the later stages of cardiomyopathy.

Interestingly, in the hearts of aging myopathic hamsters, DBH activity tended to increase in parallel with tyrosine hydroxylase activity. There was no detectable decrease in DBH activity such as postulated by Sole and Hussain [26] to explain their findings of terminal increase in dopamine and decrease in norepinephrine activity.
concentrations. In addition, cardiac dopamine was not increased in the present study although norepinephrine concentration was decreased in failing hearts. The present results, while not totally in accord with those of Sole and Hussain [26] with respect to dopamine, do not challenge the proposal that DBH activity may become rate-limiting at high levels of sympathetic activity. It is possible in the animals used in this study that sympathetic activation of heart was not yet maximal at the time of sacrifice.

It is important to note that the data on sympathetic enzyme activity are expressed in two ways, per unit weight (Fig. 3) and per total heart (Fig. 2). While enzyme activity expressed per total heart increased in myopathic as contrasted with control hearts, this change was not so evident when data were expressed per unit weight probably because of the dilutional influence of myocardial hypertrophy. Data on neurotransmitters were expressed only per unit weight of tissue and here the trends
parallel those seen with enzyme activity although again, the late decrease in norepinephrine concentration may have been partly the result of dilution by hypertrophied tissue and partly the result of net loss of neurotransmitter. The initial increase in norepinephrine concentration in the 30-day myopathic heart must represent a net increase in tissue stores of norepinephrine since the changes are directionally opposite to those which might be explained by the influence of hypertrophy. All of the sympathetic neurochemical changes are consistent with increased sympathetic activation of the myopathic heart even in the terminal stages of heart failure [24].

The new information in this study concerns the parasympathetic system and the relationship between parasympathetic and sympathetic neurochemical indices over time in hearts of myopathic hamsters. Choline acetyltransferase activity expressed in terms of unit weight of tissue (Fig. 2) might have been misleading since values would be affected by a dilutional influence of ventricular hypertrophy as mentioned above for sympathetic indices [4]. However, by expressing values in terms of total heart, the influence of hypertrophy was minimized and primary changes in choline acetyltransferase could be evaluated.

These results indicate that responses of the parasympathetic nervous system in hearts of myopathic hamsters differ qualitatively from responses of the sympathetic nervous system. Since there were disparate changes in sympathetic and parasympathetic neural indices, the basic mechanisms of the changes may relate to alterations in central neural regulation rather than to selective alterations in peripheral nerve fibers.

The time course of parasympathetic indices did not reveal early changes in the parasympathetic system. CAT activity was normal in 30-day-old myopathic hamsters and decreased significantly in 180-day-old myopathic hearts. One interpretation of these results is that neurochemical alterations may not be hereditary but rather develop as a consequence of other determinants, possibly activation of somatic reflexes [1,11].

In a study reported previously from this laboratory [18] the heart rates of myopathic hamsters approximately 180 and 300 days of age were significantly lower than the heart rates of control hamsters of corresponding age. Similar findings were reported by Ablemann et al. [2]. The present observations of increased sympathetic and decreased parasympathetic neurochemical indices in the myopathic heart at these ages do not appear to explain the lower heart rates and suggest the involvement of other factors as yet unknown.

One question about hamsters with skeletal and cardiomyopathy is the significance of increased tyrosine hydroxylase and dopamine-β-hydroxylase in the heart. Sympathetic overactivity and catecholamine-induced cardiac necrosis have been proposed as important contributing influences to the pathogenesis of cardiomyopathy, particularly the late, so-called “necrotic phase” (5–7,27). This speculation is based primarily on experiments which demonstrated that cardiac lesions resemble those induced with infusions of catecholamines to normal animals [7], that infusions of catecholamines to myopathic hamsters markedly accelerate progression of the cardiac lesions [6], and treatment with calcium and β-adrenergic receptor antagonists
prolongs the life-span and slows the progression of cardiac lesions [13]. However, the observation that heart rates are lower in myopathic hamsters suggests a need for caution in the interpretation of sympathetic neural changes. Although it is not yet possible to determine whether the high sympathetic enzyme activities represent primary pathogenetic changes or secondary compensatory changes, the early increases in dopamine-β-hydroxylase activity tend to favor a pathogenetic role.

A second question concerns the significance of the decreases in choline acetyltransferase activity in myopathic hearts. The enzyme activity appears to be excessive (10-fold greater) in relation to actual acetylcholine turnover in guinea pig heart [12,21]. If a similar relationship exists between enzyme activity and neurotransmitter turnover in hamster heart, the lower choline acetyltransferase activity might not result in altered parasympathetic neural influence in the myopathic heart. On the other hand, the fact that choline acetyltransferase activity decreases and tyrosine hydroxylase activity increases both achieved statistical significance in 180-day-old myopathic hamsters raises the possibility of impaired parasympathetic modulation of sympathetic neuronal function [14]. Future studies need to address these issues and evaluate the function of the parasympathetic system more directly. Measurements of acetylcholine levels and turnover to assess neuronal function directly may be more informative than testing heart rate responses to atropine or other pharmacologic interventions in view of the myocardial disease.

The major new finding in this study is evidence of altered cardiac parasympathetic innervation in myopathic hamsters. The possibility of a deficiency of neurotransmitter, acetylcholine, and impaired parasympathetic regulation which might contribute to augmented sympathetic stimulation and the pathogenesis of heart disease in this hamster model via catecholamine-induced cardiac necrosis is raised by these results and should be investigated in the future.

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