The Syrian Golden Hamster: A Model for the Cardiomyopathy of Friedreich's Ataxia

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SUMMARY: *In light of the available information on the cardiomyopathy of Friedreich's ataxia, the cardiomyopathic Syrian hamster may be an appropriate laboratory model. Cardiomyopathy in these animals is a result of calcium accumulation. We analyzed the atria and right and left ventricles from cardiomyopathic (CM) and random bred (RB) animals for calcium, magnesium, and iron concentrations at 30-40 and 60-70 days of age (age of maximum lesioning). There are no significant differences in the concentration of iron or magnesium among age-matched groups. The concentration of calcium in the left ventricles of the CM animals at 60 days old is 14 fold higher than that of RB animals. Although there is a significant difference in the concentration of calcium in the left ventricles of younger animals, it is not as pronounced as the difference in older animals. Analysis of the taurine concentration in 30-40 day old animals revealed that the CM animals show slightly higher taurine concentrations than RB in the whole heart. In 60 day old CM hamsters the β-adrenergic receptor density of the ventricles is unchanged. This indicates that calcium overload is not due to adrenergic supersensitivity.*

RÉSUMÉ: *Nous croyons que le hamster Syrien pourrait constituer un modèle animal adéquat de la cardiomyopathie retrouvée dans l'ataxie de Friedreich. Chez ces animaux la cardiomyopathie résulte d'une accumulation de calcium. Nous avons analysé le contenu en calcium, magnesium et fer des oreillettes et des ventricules droits et gauches de hamsters cardiomyopathiques (CM) ou contrôles (random bred; RB), et ce à 30-40 et à 60-70 jours. Il n'existe aucune différence dans la concentration de fer ou de magnésium dans les groupes apparillés pour l'âge. La concentration de calcium est augmentée de 14 fois dans les ventricules gauches des animaux CM à 60 jours, par rapport aux contrôles RB. La différence dans le même paramètre n'est pas aussi marquée chez les animaux plus jeunes. La concentration de taurine est légèrement augmentée chez les animaux CM à 30-40 jours par rapport aux animaux RB (coeur entier). A 60 jours les hamsters CM ne montrent aucune modification dans la densité des récepteurs β-adrénergiques. Ceci indique que la surcharge calcique n'est pas due à une supersensibilité adrénergique.*

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INTRODUCTION

A common feature of Friedreich's ataxia is the presence of cardiomyopathy. It appears to be an integral part of Friedreich's ataxia and not a phenomenon secondary to the disease process (Sanchez-Casis et al., 1976). The one heart autopsied by Sanchez-Casis showed severe and diffuse intracellular fibrosis, with iron deposits and areas of intracellular calcification. There are a number of studies indicating that alterations in cell calcium uptake and content are closely related to cardiac necrotic processes (Fleckenstein, 1971; Fleckenstein et al., 1974; Fleckenstein et al., 1975). Calcium overload may be caused by a number of mechanisms. Hartman and Booth (1966) have suggested that sympathetic overactivity secondary to lesion of the vagal nuclei is responsible for myocardial damage. In such a case, the cardiac muscle is abnormally sensitive to adrenergic stimulation, which thus causes an increased flux of calcium across the cell membrane. Another possible biochemical abnormality is an impairment of oxygen transportation or utilization (Malo et al., 1976). An intrinsic impairment of oxygen availability to the cell would have a synergistic effect on calcium overload.

Taurine (2-amino ethane sulfonic acid) is a β-amino acid and is transported in the brain, heart, platelets, and kidney by a system specific for β-amino acids (Chubb and Huxtable, 1978; Hruska et al., 1978; Goldman and Scriver, 1967). As taurine concentrations in the heart are several hundred times higher than in the serum, its transport into the heart is energy dependent. The level of this amino acid in the heart is altered in certain cardiac disorders. Huxtable

and Bressler (1974) have shown heart taurine content is increased in humans dying of chronic congestive heart failure. The same laboratory has shown that taurine has a modifying influence on calcium kinetics (Huxtable and Chubb, 1976). Furthermore, it appears that taurine causes an increase in the affinity of calcium for various intracellular structures (Huxtable and Bressler, 1973; Dolara et al., 1973). These authors have suggested that the pathological effects of high cellular calcium concentrations are due to the free calcium ion, and taurine protects against energy depletion and calcified deposits by holding the extra calcium in a bound form. In a recent review (Huxtable, 1978), it was suggested that taurine may be closely involved in the pathogenesis of the cardiomyopathy associated with Friedreich's ataxia.

Since the direct study of cardiomyopathy in humans has numerous and obvious limitations, we have examined cardiomyopathic states in laboratory animals to find an appropriate model system. Huxtable (1978) suggested the use of the cardiomyopathic Syrian hamster as an experimental model for the cardiomyopathy that occurs in Friedreich's ataxia. Hamsters of the Bio 14.6 strain suffer a genetic cardiomyopathy that is autosomal and recessively transmitted (Lossnitzer and Bajusz, 1973). Cardiomyopathic lesions are detectable at approximately 60 days of age (Bajusz et al., 1969). The lesions are followed progressively by cardiac hypertrophy at about 120 days of age, compensated cardiac failure at about 200 days, and full failure with typical sequellae culminating in death at an age of approximately 300 days (Schwartz et al., 1972). In these animals we have made regional ionic and taurine analyses and have tested the responsiveness of the β-adrenergic system.

MATERIALS AND METHODS

Male cardiomyopathic Syrian golden hamsters (CM) of the Bio 14.6 strain and random bred control hamsters (RB) were purchased from Telaco, Bar Harbor, Maine.

Methods:

Spectral analysis: The CM and RB animals were sacrificed by decapitation. Hearts were removed and washed in 0.9% saline solution containing glucose (1.3 g/l). Atria, right and left ventricles were dissected, washed, and blot-dried before transfer to preweighed calcium-free volumetric flasks. The samples were digested for one hour in 65% ultrapure nitric acid (EM Laboratories, Elmford, New York) on a steam bath. Sufficient ionization buffer was added to make the final solution 1000 ppm cesium. After cooling, the samples were diluted with distilled deionized water. Precipitated proteins were filtered, and the solution analyzed by conventional atomic absorption spectrophotometry with a Varian AA5. An air/acetylene flame was used for iron and a nitrous oxide/acetylene flame for calcium and magnesium. The analytical wave lengths used were 422.7, 285.2, and 248.3 nm respectively for calcium, magnesium, and iron.

Protein determination: Protein determinations for receptor binding studies of the ventricle were performed by the method of Lowry et al. (1951).

Receptor binding assay: Tissue from each heart ventricle was homogenized with a polytron homogenized (Brinkman, setting 5 for 30 sec) to make a 2.5% homogenate in 0.05 M Na^+-K^+ phosphate buffer (pH 7.4). The homogenate was passed through several layers of cheese cloth, and centrifuged at 48,000 g for 20 min in a Sorvall RC 2-B centrifuge. The supernatant was measured and discarded. The pellets were resuspended in Na^+-K^+ phosphate buffer in a volume equal to that of the discarded supernatant. The suspension was rehomogenized for 5 seconds. β-Adrenergic receptors were assayed by the method of Bylund and Snyder (1976). Tissue homogenates containing 80 μg protein were incubated for 30 min at 25°C in 2 ml of 0.05 M Na-K phosphate buffer containing 0.25 μM

TABLE 1

Regional Cation Concentrations in Hearts from 30-40 Day Hamsters

	Location	Wet tissue weight (mg)	μg/g tissue Ca	Mg	Fe
RB	Right and left atria	8.7±0.4	45±5	223±23	not detectable
	Right ventricle	31.5±1.8	68±6	253±1	45±6
	Left ventricle	124.5±4.0	40±3	233±21	41±3
CM	Right and left atria	9.4±0.5	59±11	239±12	not detectable
	Right ventricle	40.0±3.5	68±5	247±1	39±2
	Left ventricle	133.0±5.6	68±8*	246±3	51±2

N=4 All values±SEM *$p<0.025$

TABLE 2

Regional Cation Concentrations in Hearts from 60-70 Day Hamsters

	Location	Wet tissue weight (mg)	μg/g tissue Ca	Mg	Fe
RB	Left and right atria	13.4±1.0	67±6	205±4	54±2
	Right ventricle	54.2±1.0	52±2	236±6	56±1
	Left ventricle	208.0±6.5	36±0	221±3	46±2
CM	Left and right atria	14.0±1.5	81±8	197±1	61±7
	Right ventricle	43.8±1.0	63±4	222±2	56±2
	Left ventricle	190.0±5.8	510±93**	217±5	47±0

N=4 All values±SEM **$p<0.005$

^{3}H-dihydroalprenolol (DHA; 58 Ci/nmole, NEN) in the presence and absence of 0.1 μM (-)-propranolol. The reaction was terminated by vacuum filtration through GF/B glass fiber filters followed by four 5 ml rinses with buffer kept at 25° C. Bound ^{3}HDHA retained on the filter was extracted in 9 ml of a toluene based scintillation cocktail and radioactivity counted. Specific ^{3}H-DHA binding was defined as the binding displaceable by 0.1 μM (-)-propranolol.

Taurine analysis: Animals were sacrificed by decapitation and the heart removed, washed in saline, and dissected into areas. After being transferred to preweighed centrifuge tubes, the tissues were diluted 1:10 with3.5% 5-sulfosalicylic acid, sonicated to homogenity, centrifuged, and the supernatant analyzed for taurine.

RESULTS

There are no significant alterations in the concentrations of Fe and Mg in the atria, right or left ventricles of CM compared to RB hamsters at either 30 or 70 days of age. However, the calcium concentration of the left ventricle increases with age in CM relative to RB hamsters (Tables 1 and 2). Left ventricular calcium concentration is increased 70% in 30-40 day old animals, and is increased 1300% by 60-70 days of age. There are no corresponding changes in the right ventricle. In RB hamsters, the calcium concentration of the right ventricle is higher than that of the left at both age ranges (Table 1 and 2).

CM hearts show a moderate but significant increase ($p<.05$) in taurine concentration (Table 3). Regional analyses indicate that the right ventricle is the only area not showing an increased concentration relative to heart from RB hamsters (Table 4).

Analysis of a small number of hearts indicates that the development of hypertrophy is not associated with alteration in β-adrenergic receptor density (Table 5, Bmax unchanged). However, the affinity for ^{3}H-dihydroalprenolol may be decreased in CM hamsters. This altered affinity also is indicated by a Scatchard plot (Fig. 1). These data are based on only one RB and two CM hamsters.

DISCUSSION

Our study has shown a substantial increase (14 fold) in the concentration of calcium in the left ventricles of CM animals versus RB ones at 60-70 days. A less pronounced but statistically significant increase in calcium concentrations is also present at 30-40 days. Taurine concentrations are elevated in the atria and left ventricle in 30-40 day old CM hamsters. Our results, based on a limited study, do not indicate that supersensitivity of the β-adrenergic system is responsible for the calcium overload.

A decrease in the concentration of magnesium and an increase in the concentration of iron occurs in CM hamsters between ages of 30 and 60 days. However, when each age group is compared to its appropriate control group, such alterations are not

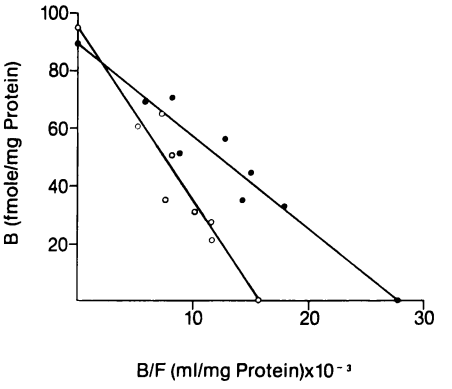

Fig. 1 — Scatchard plot of [^{3}H]-dihydroalprenolol binding to ventricular protein.
B = bound [^{3}H]-dihydroalprenolol (fmole/mg protein)
F = free [^{3}H]-dihydroalprenolol (fmole/ml)
• RB hamster ○CM hamsters (mean of two preparations).

TABLE 3

Taurine Concentration in Hamster Hearts (30-40 Days)

		Animal weight (g)	Heart weight (mg)	Taurine (μmole/g wet tissue)
RB	(N=9)	58.6±2.9	172±5	24.9±1.6
CM	(N=8)	62.4±2.6	183±7	30.4±1.8*

*$p<0.05$ All values±SEM

TABLE 4

Regional Concentration of Taurine in Hamster Hearts (30-40 Days)

	Taurine (μmole/g wet tissue) RB	CM
Right atrium	26.4±3.3	36.8±9.3
Left atrium	26.7±1.7	33.5±1.0**
Right ventricle	36.3±5.9	35.0±2.0
Left ventricle	26.9±3.6	29.4±1.8
Total Taurine	28.5±2.3	30.8±2.7

N=4 **$p<0.025$ All values±SEM

TABLE 5

β-Adrenergic Receptor Binding In Heart Ventricles (60 Days)

	Ventricular weight (mg)	Protein (mg/ml)	Bmax (fmole/mg protein	Bmax (nmole/ventricle)	K_d (nM)
RB	222.0	1.61	89.0	5.55	0.257
CM	260.4	1.58	95.0	5.85	0.477

These data are derived from one RB and two CM hearts.

significant. Therefore, we believe such alterations to be an age-dependent phenomenon and are not directly involved in the cardiomyopathy.

The cardiomyopathy in these hamsters seems to be calcium dependent. Calcium overload may lead to cardiomyopathy in hamsters by activation of calcium dependant ATPases, leading to energy depletion; by impairment of energy production at the cellular level, or by increased calcium content not accompanied by comparable taurine alterations, leading to increased concentrations of free (energy depleting) calcium ions.

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