A QUANTITATIVE AND QUALITATIVE DEFECT IN THE SARCOPLASMIC RETICULUM IN THE HEREDITARY CARDIOMYOPATHY OF THE SYRIAN HAMSTER*

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Summary:

In the Syrian Hamster with a cardiomyopathy characterized by ventricular dilitation, compensatory hypertrophy and congestive failure, the fragmented sarcoplasmic reticulum (SR) has been isolated. In normal (N) and myopathic (CM) hamsters SR has been studied relative to rate of ATP dependent calcium trapping, calcium capacity per unit SR, and SR quantity per unit of myocardium. The ATP dependent Ca^{++} oxalate pumping of the SR was not different from N in CM at 10 days, reduced 22.4% at 200 days, 30.5% at 300 days with moderate failure, and 77.5% at 300 days with severe failure. The quantity of SR in the tissue as judged by the Ca^{++} oxalate capacity of homogenate was unchanged at 10 days but significantly reduced at 200-300 days. Nevertheless the Ca^{++} oxalate capacity per unit SR was unchanged at all ages studied, suggesting a dilution of the SR by the resultant hypertrophy.

Under physiologic conditions the sarcoplasmic reticulum assumes a major role in supplying calcium for contraction, while the ATP dependent calcium pump of the sarcoplasmic reticulum is thought to bring about relaxation by sequestering the calcium from the myofibrils (1,2,3). In previous studies it was suggested that a depression of the sarcoplasmic reticular ATP dependent calcium pump may play a role in the etiology of myocardial failure (4,5). It has been theorized that were this pump to be depressed, it would pump less calcium per unit of time, leading to less calcium in the sarcoplasmic reticulum and eventually to less calcium for contraction.

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Although no direct cause and effect relationship has been demonstrated, certain drugs such as barbiturates, local and general anesthetics which cause depression of contractility of cardiac and skeletal muscles (6,7,8) have been shown to depress the ATP dependent calcium pump of skeletal and/or cardiac sarcoplasmic reticulum (1,4,9,10,11). Further, in acute heart failure in dogs (5) and chronic heart failure in cattle (12) and humans (13) a depression of the ATP dependent calcium pump of the sarcoplasmic reticulum has been demonstrated.

The BIO 14.6 strain of Syrian hamster offers a unique opportunity to study the function of the sarcoplasmic reticulum in a naturally occurring form of myocardial failure (14). These animals have been bred with an autosomal recessive gene that produced a spontaneous cardiomyopathy. Myolytic lesions with minimal inflammatory reaction occur between the 30-40th day of life and reach their peak at approximately 60 days (15). Thereafter, healing occurs with minimal proliferation of connective tissue. At approximately this time, hypertrophy develops and continues at varying rates. By 200 days, hypertrophy is well advanced and signs of congestive heart failure begin to appear characterized by marked fluid accumulation with ascites and pleural effusions and hepatomegaly.

With these findings in mind, BIO 14.6 hamsters and appropriate normal controls have been studied with specific attention to the quantity of sarcoplasmic reticulum in these hearts, and the quality or performance of the sarcoplasmic reticulum as characterized by the calcium oxalate capacity and calcium oxalate rates at different stages of the disease.

Methods:

Animals were sacrificed either by decapitation with a guillotine or by

cervical fracture. The hearts were quickly removed and placed in ice cold 0.3 M sucrose and 10mM Imidazole (pH 7.0). The hearts were trimmed of great vessels and atria, minced and weighed. Homogenization was carried out in a Sorvall Omnimixer with a setting of 120 on a powerstat reostat for 40 seconds. Blending was done in a 4:1 volume: weight ratio of 0.3 M sucrose and 10mM Imidazole (pH 7.0). Isolation of the fragmented sarcoplasmic reticulum was carried out using the procedures previously reported (9) except centrifuges and rotors used were from International Equipment Company. The Millipore filtration technique previously described was used for measuring fragmented sarcoplasmic reticular rates and capacity (9). When capacity was being determined .64 mM Ca Cl_2, 1.6 mM EGTA, 6 mM creatine phosphate and 5mM Azide were included in the incubation bath. All experiments were carried out at 37° C. The technique of Solero, Gertz and Briggs (16) was used for estimating the quantity of sarcoplasmic reticulum in the myocardium. Proteins were determined by the method of Lowry et al (17).

Results:

The failing hearts utilized in this study were obtained from animals with a naturally occurring cardiomyopathy. Neither surgical interventions (12) or pharmacological agents (13) which might produce changes in and of themselves have been introduced.

The rate of ATP dependent calcium pumping of the fragmented sarcoplasmic reticulum averaged 1.08 ± 0.06 (± SEM) μmoles/mg FSR protein/min in normals of all ages studied (Table I). In the myopathic animals, the rate was unchanged from the normals at 10 days. However it was reduced by 22.4% ± 0.96% ($P < .05$) at 200 days, reduced by 30.5% ± 1.0% ($P < .05$) at 300 days when the animals were in moderate congestive failure and by 77.5% ± 1.0% ($P < .05$) at 300 days when

Table I

SARCOPLASMIC RETICULUM

Calcium Pumping Rate (2)

	Normal [1]	Myopathic [1]
10 days	1.16 ± .032 [3]	1.09 ± 0.044
200 days	1.07 ± .061 (P .05)	.837 ± .023
320 days [4]	1.12 ± .066 (P .05)	.750 ± .023
300 days [5]	1.04 ± .041 (P .05)	.243 ± .018

1 Each value represents the mean of 3 experiments with 10-35 animals in each experiment.

2 μmole/mg FSR protein/min

3 ± ISE ± standard error of the mean

4 Compensated.

5 Uncompensated Congestive Heart Failure.

the animals were noted to be in severe congestive failure. Death in congestive heart failure generally ensues at this period of time. The 200 day old myopathic hamsters had congested livers, but no ascites or obvious peripheral edema. The ventricles in these animals weighed 1.5-1.75 times that of appropriate controls. The quantity of sarcoplasmic reticulum in the tissues as judged by the calcium oxalate capacity of the homogenate in the presence of azide was unchanged at 10 days but was reduced by approximately 50% per unit weight of heart when compared with appropriate controls (Table II). Nevertheless, calcium oxalate per unit of fragmented sarcoplasmic

Table II

CALCIUM CAPACITIES

	Whole Heart [1]		Sarcoplasmic Reticulum [3]	
	Normal	Myopathic	Normal	Myopathic
10 days	43.4 [2] ± .404 [3]	42.1 ± .403	5.30 [6] ± .153	5.73 ± .291
200 days	42.4 ± .393 (P .05)	22.2 ± .755	5.80 ± .203	5.77 ± .240
300 days [4]	42.9 ± .825 (P .05)	21.2 ± .775	5.80 ± .379	5.73 ± .376
300 days [5]	42.4 ± .731 (P .05)	21.5 ± .517	5.80 ± .239	5.50 ± .260

[1] Each value represents the mean of 3 experiments with 10-35 animals in each experiment.

[2] μmole/gm wet wt. of heart.

[3] ± ISE

[4] Compensated.

[5] Uncompensated Congestive Heart Failure.

[6] μmole/mg FSR protein.

reticulum was unchanged in myopathic hearts as compared to controls (Table III).

Discussion:

Briggs et al (4) have suggested that a depression of the sarcoplasmic reticular calcium pump might be casually related to myocardial failure. This hypothesis was based on the direct action of various pharmacological agents to depress calcium pumping by preparations of sarcoplasmic reticulum. Other

studies of the spontaneously failing heart lung preparations lent further support to this view (5). Recently others have shown a similar depression of the ATP calcium pumping in association with myocardial failure. One of these studies (12) was performed on hearts obtained from animals after surgical interventions and the other (13) on human hearts obtained only after multiple pharmacological interventions.

The present study provides substantial support for the view that there is a depression of the rate of calcium pumping by the sarcoplasmic reticulum in the hereditary cardiomyopathy developing of the Syrian hamster and that this depression increases as the evidence of congestive heart failure progresses. However the pertinence of these observations to the etiology of heart failure in clinical situations remains speculative. While this form of a cardiomyopathy may not be representative of all forms of myocardial failure, the consistent nature of the defect in association with reduced myocardial function provides an intriguing model.

As myocardial dilation and failure develops in these animals, hypertrophy occurs. The calcium oxalate capacity of the fragmented sarcoplasmic reticulum per milligram of reticular protein remains normal thoughout; this is true although there is a reduction in the rate of calcium pumping. However the calcium oxalate capacity of homogenates of the whole heart are reduced, attesting to the fact that the quantity of sarcoplasmic reticulum per unit of muscle mass is reduced. Thus, during the hypertrophy in the present pathological condition there appears to be a "dilution" of sarcoplasmic reticulum by the by the hypertrophying myofibrillar elements. Were this the case, one would reach a point where there would be an inadequate amount of sarcoplasmic reticulum to store the calcium needed to activate the myofilaments and failure might ensue. Thus, in this cardiomyopathy, in addition to a qualitative defect in calcium pumping reflected by reduced rates,

there appears to be a quantitative reduction in the calcium capacity per unit of muscle due to dilution of the sarcoplasmic reticulum by hypertrophy.

Studies are now underway to further elucidate the sequence of events that occur in this cardiomyopathy and characterize the non oxalate ATP dependent calcium binding and calcium stimulated "extra" ATPase activity of the sarcoplasmic reticulum. Moreover, the question as to whether the apparent "dilution" of the SR by myofibrillar hypertrophy occurs in other forms of compensatory hypertrophy is presently being explored. Were this to be the case, it might help to explain the reduction in myocardial contractility which has been found in the presence of right ventricular hypertrophy in cats with experimental pulmonary artery stenosis (18).

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