ACTA STEREOL 1985; 4/2: 217—222
PROC ESS IV-1 GÖTEBORG 1985
ORIGINAL SCIENTIFIC PAPER

ULTRASTRUCTURAL MORPHOMETRIC STUDY ON THE RAT HEART IN CHRONIC ALIMENTARY THIAMINE DEFICIENCY

Gerhard Mall, Hans-Jörg Möbius, Torsten Mattfeldt, Rainer Leonhard

Institute of Pathology, University of Heidelberg, Im Neuenheimer Feld 220/221, D-6900 Heidelberg, F.R.G.

ABSTRACT

Male Wistar rats were fed with a thiamine-free diet for 35 days. The growth of the thiamine-deficient animals was considerably suppressed, as compared to a pair-fed control group (body weights: 140.4 ± 4.3 g versus 243.1 ± 4.3 g). A weight-matched control group was, therefore, evaluated in addition. Histologic and ultrastructural investigations of the hearts did not reveal any morphologic abnormalities. Morphometric analysis was performed on left ventricular papillary muscles and disclosed significant differences of stereologic parameters ("densities") between the thiamine-deficient group and the control group, but no differences were detected between the thiamine-deficient group and the weight-matched control group. It is concluded that stereologic parameters ("densities") of the myocardium depend on the body weight and on the heart weight, respectively, and that our experimental model of thiamine-deficiency was associated with a normal structure of the myocardium.

Keywords: Myocardium, stereology, thiamine deficiency, ultrastructure.

INTRODUCTION

Human beriberi heart disease is caused by cardiovascular effects of thiamine deficiency (Aalsmeer and Wenckebach,1929). Morphologic studies on the myocardium of thiamine-deficient rats revealed focal areas of myocardial fiber necrosis as well as mitochondrial abnormalities (Bozner et al.,1969; Davies and Jennings, 1970).

The present study was designed to analyse the quantitative morphologic reaction pattern of the myocardium in chronic alimentary thiamine deficiency. In contrast to earlier studies,fixation artifacts which might resemble pathological changes were to be prevented by vascular perfusion with the fixative.

EXPERIMENTAL PROCEDURE

Eighteen male Wistar rats (average body weight: 57.4 ± 5.3 g (SD)) were randomly divided into two groups. Nine rats were fed with a special diet free of thiamine (ALTROMIN) for 35 days ad libitum. Nine rats were pair-fed with a standard diet (ALTROMIN). All rats were caged individually. Coprophagy was excluded by the use of special cages. Body weights were determined weekly.

The viscera were fixed by retrograde vascular perfusion at a pressure of 90 mmHg. Before fixation the vascular system was flushed with a dextran solution containing procaine-HCl for 2 minutes which improves the microcirculation and leads to cardiac arrest in diastole. The vena cava inferior was

incised to drain the blood. The incision was performed 10 seconds after the start of the dextran infusion. This procedure may avoid the collaps of capillaries caused by low venous pressures. After fixation left ventricular papillary muscles were randomly cut either longitudinally or transversely with a tissue sectioner (Mattfeldt and Mall, 1984).

All specimens were postfixed in 1% OsO_4, dehydrated in ethanol and embedded in EPON - ARALDITE. Semithin sections (1 µm) were stained with methylene blue and basic fuchsin and examined by light microscopy using oil immersion and phase contrast. Ultrathin sections were stained with uranylacetate and lead citrate and examined with a ZEISS EM 10 electron microscope.

The growth of the thiamine deficient rats was considerably suppressed (body weights: 140.4 ± 4.3 g compared to 243.1 ± 4.3 g). A weight-matched control group (n = 9) was, therefore, evaluated in addition (body weights: 138.3 ± 5.7 g).

Volume densities (V_V) were obtained by point counting using the equation

$$V_V = P_P \qquad (1)$$

Length densities (L_V) and surface densities (S_V) were calculated according to the following stereologic equations:

$$L_V = c_1(K_L\alpha)\ Q_A \quad \text{(capillaries)} \qquad (2)$$

$$S_V = c_2(K_S\alpha)\ B_A \quad \text{(capillaries)} \qquad (3)$$

$$S_V = 4/3\pi\ (B_{A1} + 2\ B_{A2}) \quad \text{(mitochondria)} \qquad (4)$$

$$S_V = 4/\pi\ B_A \quad \text{(inner mitochondrial membranes)} \qquad (5)$$

From (2) and (3) we obtain model-based L_V and S_V estimates of anisotropic tubular structures (based on a Dimroth-Watson orientation distribution (Mattfeldt and Mall,1984; Weibel,1980)). The c values depend on the sectioning angle α and the concentration parameter K . Estimates of K values are derived from the ratio of counts on transverse and longitudinal sections.
From (4) we obtain a model-based S_V estimate of anisotropic tubular structures (based on a Marriott distribution),provided that the degree of anisotropy is low ($B_{A1}/B_{A2} \leq 6/5$; B_{A1} and B_{A2} are the B_A values on transverse and longitudinal sections, respectively) (Weibel, 1980).
Equation (5) is valid in the case of randomly oriented surfaces independent on the sectioning angle α.

The numerical density (N_V) of muscle cell nuclei was obtained by the equation:

$$N_V = (N_A)\ \mu^{-1} \qquad (6)$$

The mean caliper length µ was estimated by serial sectioning without reconstruction as proposed by Cruz-Orive (1980). 30 serial transverse sections (thickness:1.5 µm) per animal were used to determine the mean caliper length of nuclei along the axis of the papillary muscle (15 - 20 nuclei per animal).

The stereologic analysis was performed as a multistage sampling procedure.

Stage 1 (magnification 1,020 : 1, light microscopy): Three random transverse sections per animal and eight test areas per section (total test area: 58,000 μm^2) were analysed with a ZEISS eyepiece containing 100 points and 10 lines (total length: 927 µm). Test areas were obtained by systematic subsampling. The points were used for point counting, the test lines to obtain intersections with the profile contours to estimate the B_A values. The following densities in space were derived from counts at this stage: V_V, L_V, and S_V capillaries, and N_V myocardial cell nuclei. Reference volume: myocardial tissue.

Stage 2 (magnification 32,500 : 1, electron microscopy): One random transverse ultrathin section per animal was used to determine V_V mitochondria, V_V myofibrils and V_V sarcoplasmic matrix. Ten random test areas with 80 test points per area were obtained by systematic subsampling (distance between 2 points: 0.5 µm). Counting was performed on a television monitor. B_A values of mitochondria were measured on-line with a semiautomatic image analysing system (VIDEOPLAN, KONTRON) on one longitudinal section (15 test areas) and on one transverse section (10 test areas). Reference volume: myocardial cell.

Stage 3 (magnification 145,700 : 1, electron microscopy): One random ultrathin section per animal was selected to determine S_V inner mitochondrial membranes. Fifteen systematically subsampled test areas were evaluated and estimates of S_V were derived from intersections with a square lattice containing 63 squares (side length: 0.2 µm) via the B_A values. The "loss" of obliquely cut membrane images was corrected as described elsewhere (Mall et al., 1977). Reference volume: myocardial cell.

RESULTS

The growth of the thiamine-deficient rats was considerably suppressed (tab.1), but light and electron microscopic investigations of the hearts failed to reveal morphologic abnormalities (fig.1). Focal necroses of myocardial fibers were not observed. The relative heart weights (heart weight/body weight) were lower in the control group than in the thiamine-deficient group and the weight-matched control group, but a significant difference between the thiamine-deficient group and the weight-matched control group could not be established (tab.1). Furthermore, the morphometric analysis of left ventricular papillary muscles did not show significant effects of thiamine depletion. The significant differences detected by one-way analysis of variance were not associated with significant linear contrasts between the thiamine-deficient group and the weight-matched control group (tab.1). Thus, relative heart weights and morphometric variables of the heart are likely to depend on the body weight. The numerical density (N_V) of myocardial cell nuclei and the length density (L_V) of capillaries were lower and the volume density (V_V) of capillaries and the surface-to-volume ratio of mitochondria were higher in the control group than in the thiamine-deficient group and in the weight-matched control group, respectively (fig. 2, tab.1).

Table 1. Heart weights and stereological parameters of the myocardium in chronic thiamine deficiency

Parameter	Thiamine-deficient group (n=9)	Control group (n=9)	Weight-matched control (n=9)	Stat.
Body weights (g)	140 ± 4+	243 ± 4	138 ± 6	p<0.001
Relative heart weights (x 1000)	4.43 ± 0.34+	3.68 ± 0.34	4.12 ± 0.27	p<0.001
Capillaries				
L_V (mm/mm^3)	3,936 ± 265+	3,612 ± 273	4,129 ± 156	p< 0.001
S_V (cm^2/cm^3)	759 ± 73	716 ± 68	725 ± 49	N.S.
V_V (cm^3/cm^3)	0.085 ± 0.026+	0.105 ± 0.012	0.087 ± 0.011	p< 0.02
Myocardial cell nuclei				
N_V ($1/mm^3$)	77,155 ± 9,082+	62,246 ± 8,916	87,780 ± 15,320	p<0.01
V_V (cm^3/cm^3)				
mitochondria	0.266 ± 0.018	0.262 ± 0.016	0.280 ± 0.018	N.S.
sarcoplasm	0.119 ± 0.025	0.111 ± 0.013	0.130 ± 0.015	N.S.
myofibrils	0.615 ± 0.035	0.627 ± 0.026	0.591 ± 0.028	p<0.05
S_V (m^2/cm^3)				
mitochondria	2.15 ± 0.19+	2.38 ± 0.14	2.22 ± 0.19	p<0.05
inner mito. membranes	13.60 ± 1.92	13.82 ± 1.68	14.08 ± 1.22	N.S.
S_V-ratio (m^2/cm^3)				
mitochondria	8.07 ± 0.37+	9.04 ± 0.16	7.90 ± 0.13	p<0.001
Length of sarcomeres (µm)	1.96 ± 0.08	1.97 ± 0.19	2.02 ± 0.13	N.S.

Means ± standard deviations.

The means of the three groups were tested by one way analysis of variance. A result was considered to be significant if p<0.05 (Stat.) Significant linear contrasts (SCHEFFE's test) between the thiamine-deficient group and the weight-matched control group were not detected (p>0.05). + indicate significant differences between the thiamine-deficient group and the control group.

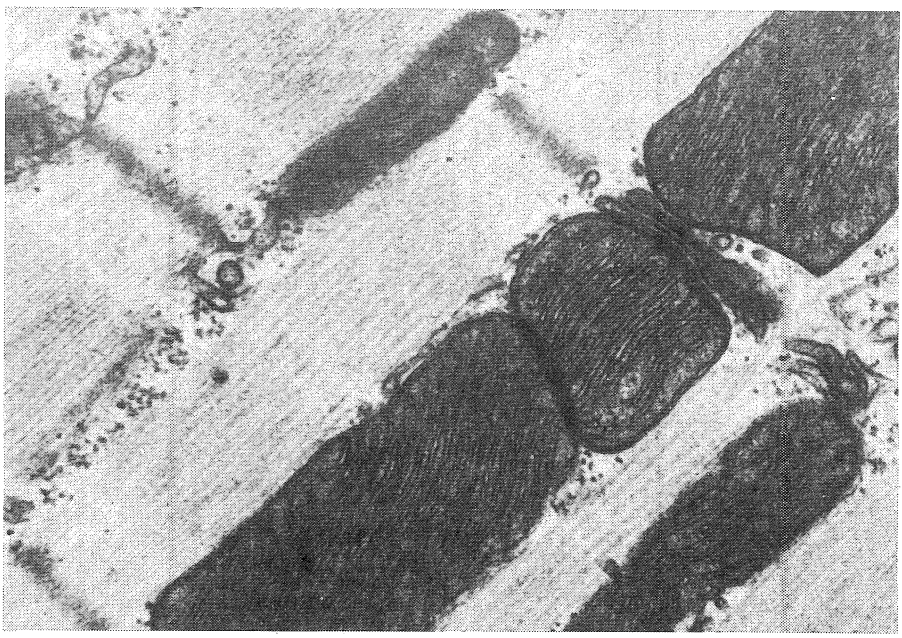

Fig. 1. Left ventricular myocardium in chronic thiamine deficiency (Final magnification 54,600 : 1). Normal appearance of myofilaments, mitochondrial membranes, T tubules and sarcoplasmic reticulum.

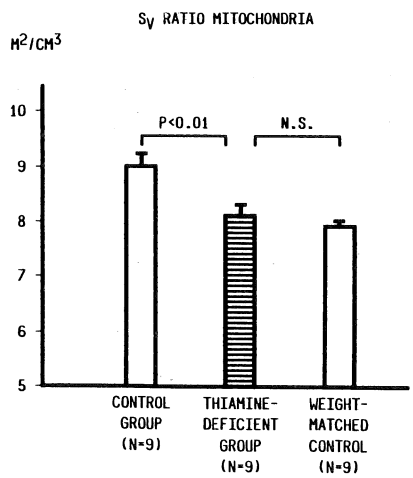

Fig. 2. The surface to volume ratios of mitochondria are significantly higher in the control group compared to the values in both, the thiamine-deficient group and the weight-matched control group. (Arithmetic means and standard deviations).

DISCUSSION

Our experiment on chronic alimentary thiamine deficiency is the first study in which 1) the hearts were fixed by vascular perfusion , and 2) a weight-matched control group was investigated in addition to the pair-fed control group. The weight-matched control group was included, since body weights were considerably changed in chronic thiamine depletion.

The stereologic parameters ("densities") did not show significant differences between the thiamine-deficient group and the weight-matched control group, but differences were found between the thiamine-deficient group and the control group.

In normal growth, the heart weight increases by enlargement of muscle cells without hyperplasia of muscle cell nuclei which results in decreased numerical densities of nuclei. Furthermore, length densities of capillaries decrease because of the well-known fact that the enlargement of muscle cells is not accompanied by a proportional increase of the number of capillaries. The higher volume density of capillaries which we observed may indicate that the size of capillaries increases during growth. At the ultrastructural level, however, the high surface to volume ratio of mitochondria in the control group cannot be derived from the enlargement of muscle cells during growth. It may be related to physiologic or biochemical changes in the growing heart.

We conclude:

1. Our experimental model of chronic alimentary thiamine deficiency was associated with a normal structure of the myocardium. All significant differences of the stereologic parameters between the thiamine deficient group and the control group are to be related to body weight or heart weight changes.
2. Stereologic parameters ("densities") of the myocardium depend on the heart weight. This fact should be considered in those experimental conditions which are associated with body weight or heart weight changes.

ACKNOWLEDGEMENT

The study was supported by a grant of the DFG (Ma 912/1-1).

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