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DIFFERENT STEREOLOGICAL REACTION PATTERNS OF THE MYOCARDIUM IN HYPERTROPHY INDUCED BY EXERCISE AND PRESSURE OVERLOAD

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ABSTRACT

Mild cardiac hypertrophy was induced in young female rats by an exercise model and in young male rats by surgical stenosis of the left renal artery. Two randomly selected transverse and longitudinal sections per animal of the left ventricular papillary muscles were studied after fixation by vascular perfusion. Estimates of length density and surface density were corrected for partial anisotropy by means of the Dimroth-Watson orientation distribution. In both models the myocardial cells showed an increase of mean cross-sectional area with unchanged quantitative cytoplasmic ultrastructure. At exercise induced hypertrophy V_V, S_V and L_V of capillaries per tissue volume remained unchanged, whereas all these estimates were significantly decreased at hypertrophy induced by pressure overload. Consequently 3D capillary-fiber ratio was increased at exercise-induced hypertrophy only. The result is discussed as capillary proliferation incited by increased myocardial perfusion at exercise.

Keywords: Anisotropy, capillaries, exercise, heart, morphometry, stereology.

INTRODUCTION

An increase of heart size due to growth of its differentiated cells (hypertrophy) can be induced by physical training and chronic pressure overload. The aim of the present study was to compare the different stereological reaction patterns of the myocardium in experimental cardiac hypertrophy induced by physical training and pressure overload.

PRACTICAL METHODS

For Model I: Hypertrophy induced by physical training, 20 young female Sprague-Dawley rats were randomly assigned to 2 groups. 10 animals performed an exercise program of 18 weeks duration with gradually increasing intensity on a motor-driven running device with exact speed control. In the final phase of the experiment the animals exercised 90 min/day at a speed of 32 m/min. 10 animals served as sedentary controls. For Model II: Hypertrophy induced by pressure overload, 44 young male Sprague-Dawley rats were randomly assigned to 4 groups. In 10 animals renal hypertension was established by surgical stenosis of the left renal artery, 12 animals served as sham-operated controls; the remaining 22 rats (assigned to different treatments) are not considered further in this investigation. The duration of the study was 8 weeks. Final blood pressure amounted to 172 $\pm$ 8 in the hypertensive group vs. 91 $\pm$ 1 mm Hg in the control group ($P < 0.001$). At the end of the experiments the animals were fixed by retrograde vascular perfusion with 3% phosphate-buffered glutaraldehyde via the abdominal aorta under chloralhydrate anaesthesia.

STEREOLOGY

Mammalian myocardium is a tissue built up nearly exclusively by a network of partially anisotropic tubular structures (muscle fibers and capillaries). The axis of anisotropy is known and can be determined macroscopically only in the papillary muscles, whereas in the free chamber walls both degree and direction of anisotropy are unknown. While the estimation of volume densities, V_V, is feasible regardless of anisotropy properties by point-counting methods, estimation of surface and length densities (S_V and L_V) requires more involved stereological techniques. The basic solutions are 1. the parametric approach, in which a specific directional model is assumed so that estimation is possible from probes of fixed directions, e.g. transverse and longitudinal sections; 2. the non-parametric approach, where unbiased estimates are obtained from the classical equations $L_V = 2Q_A$ and $S_V = 4/\pi\ B_A$ using probes with isotropic uniform random (IUR) orientations (Cruz-Orive et al., 1983; Mathieu et al., 1983; Mattfeldt and Mall, 1984; Mattfeldt et al., 1985). In the present study the parametric approach with restriction of the sampling universe to the papillary muscles was chosen, making use of the fact that the Dimroth-Watson distribution adequately describes the capillary orientation pattern of the myocardium (Mattfeldt and Mall, 1984). Accordingly, 2 randomly selected transverse and longitudinal slices (thickness: 200 µm) per heart from the left ventricular papillary muscles were embedded in Epon-Araldite, as previously described. Semithin sections (nominal thickness: 1 µm) and ultrathin sections from this material were used for the stereological evaluation which was performed as a multiple-stage sampling procedure.

At Stage I (Light microscopy, 1µm Epon-Araldit sections, final magnification 1000x) volume densities (V_V) of myocardial cells, capillaries, and interstitial tissue per tissue volume were estimated by point counting. Surface density (S_V) and length density (L_V) of capillaries per tissue volume were estimated with correction for partial anisotropy by means of equations based on the Dimroth-Watson orientation distribution, as previously described (Cruz-Orive et al., 1983; Mathieu et al., 1983; Mattfeldt and Mall, 1984). Systematic subsampling of 10 visual fields per section was performed (Fig. 1, 2).

At Stage II (Electron microscopy, ultrathin sections, final magnification 4000x) L_V of myocardial cells per tissue volume was estimated from 10 systematically selected fields per longitudinal and transverse section using the same anisotropy model. As we found that the relative bias due to partial anisotropy was negligible for myocardial cells in the left ventricular papillary muscles (< 1%), anisotropy constants for myocardial cells are not displayed in Table 1. In agreement with other groups we found that electron microscopic resolution is imperative for counting of myocardial cell profiles in hearts fixed by vascular perfusion because the width of the intercellular spaces lies below the resolution of light microscopy (Loud et al., 1984; compare Fig. 1 and Fig. 3).

At Stage III (Electron microscopy, ultrathin sections, final magnification 41460x) V_V of myofibrils and mitochondria per volume of myocardial cell cytoplasm were estimated by point counting on 30 systematically subsampled fields on 2 transverse sections per animal.

Unbiased estimates of the mean "true" cross-sectional area of myocardial myocardial cells and capillaries were obtained from the ratio V_V/L_V (Mathieu et al., 1983), and "true", three-dimensional capillary-fiber ratios were estimated from the ratio [L_V(capillaries/tissue volume)]/[L_V(myocardial cells/tissue volume)].

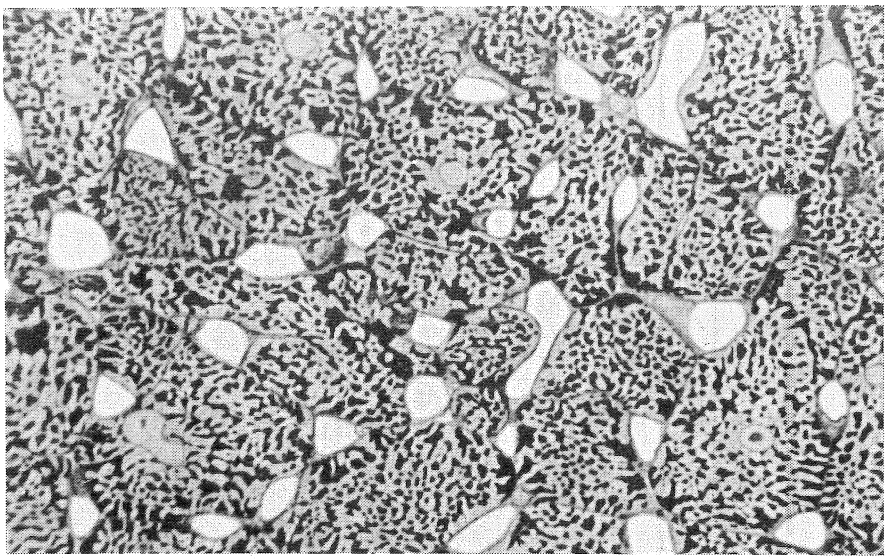

Fig. 1. Light micrograph of a transverse section of a left ventricular papillary muscle. Capillary profiles are easily delineated, whereas muscle cell boundaries cannot be distinguished with certainty. Semithin section, Toluidin-Blue. Final magnification 1050x.

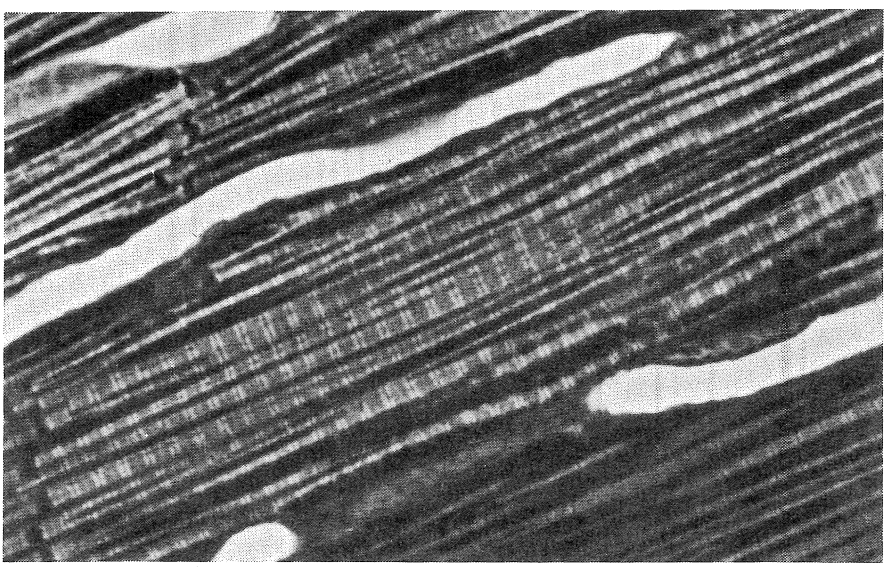

Fig. 2. Light micrograph of a longitudinal section of a left ventricular papillary muscle. Inconspicuous arrangement of capillaries and muscle fibers which show normal sarcomeres and intercalated discs. Semithin section, Toluidin-Blue. Final magnification 1680x.

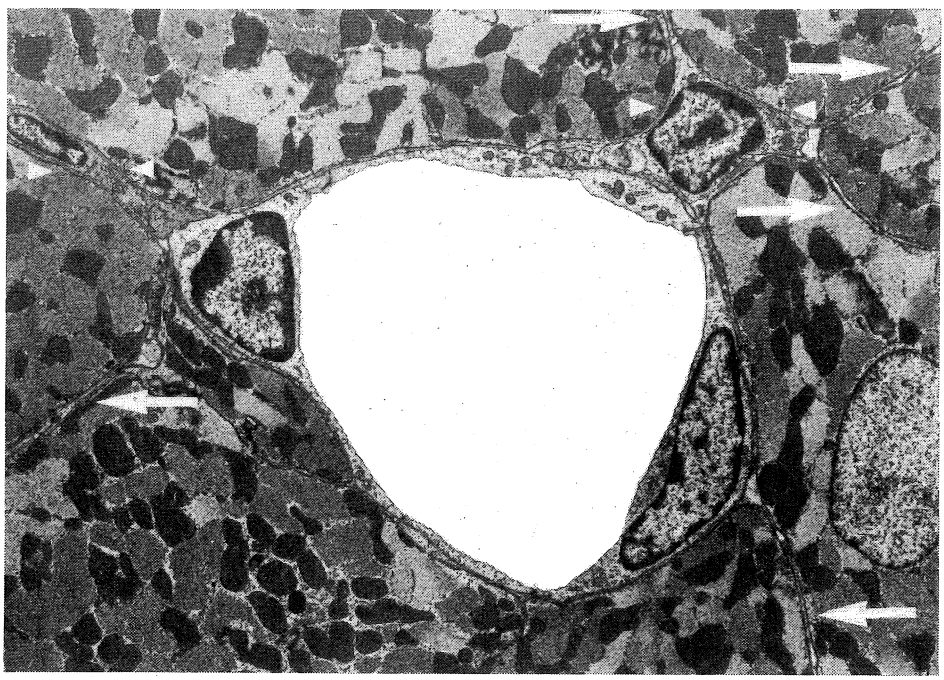

Fig. 3. Electron micrograph of a transverse section of a left ventricular papillary muscle. A capillary with 2 endothelial cells is accompanied by interstitial cells (arrowheads). The arrows indicate boundaries between muscle cells, clearly discernible at the electron microscopical level only. Final magnification: 14280x.

RESULTS

The results are displayed in Table 1. Both models lead to a mild hypertrophy of the left cardiac ventricle. The effect is mainly brought about by an increase of the total volume of the myocardial cells, which account for nearly 90% of total tissue volume. The changes of myocardial cell L_V and mean cross-sectional area are compatible with the assumption of harmonic cell growth into all 3 dimensions. There is no significant alteration of myocardial cell ultrastructure. Thus, myocardial cell reaction is very similar in both models. However, there are striking differences with respect to the capillary changes. At exercise-induced hypertrophy capillary V_V, S_V and L_V remain virtually unchanged, whereas pressure overload induced hypertrophy leads to a small decrease of capillary V_V and considerable decreases of capillary S_V and L_V. Consequently the 3-dimensional capillary-fiber ratio is increased at exercise-induced hypertrophy but not at pressure overload induced hypertrophy. None of the models alters mean capillary cross-sectional area. Small differences between the C/EX and C/PO groups are probably due to biological variability and sex difference between experiments.

Table 1. Stereological reaction patterns of myocardial hypertrophy.

	C/EX (n=10)	H/EX (n=10)	C/PO (n=12)	H/PO (n=10)
1. BASELINE DATA				
Final body weight (g)	284 ± 5	291 ± 5	287 ± 8	267 ± 5
Left ventricular weight(mg)	769 ± 21	924 ± 23***	731 ± 22	991 ± 36***
Right ventricular weight (mg)	204 ± 5	239 ± 6***	153 ± 7	180 ± 8*
2. STEREOLOGY OF CAPILLARIES AND MYOCARDIAL CELLS				
Volume of myocardial cells/ total tissue volume (%)	86.8 ± 0.5	86.9 ± 0.4	89.7 ± 0.4	91.0 ± 0.4*
Volume of capillaries/total tissue volume (%)	10.6 ± 0.4	10.2 ± 0.5	8.8 ± 0.4	7.2 ± 0.4*
Surface area of capillaries per tissue volume (mm^2/mm^3)	68.2 ± 1.8	66.8 ± 1.3	64.1 ± 1.5	51.1 ± 1.9***
Length of capillaries per tissue volume (mm/mm^3)	3744 ± 75	3803 ± 115	3694 ± 68	2978 ± 98***
Anisotropy constant of capillary axis orientation distribution	+4.8 ± 0.6	+5.5 ± 0.9	+5.7 ± 0.2	+4.1 ± 0.4**
Length of myocardial cells per tissue volume (mm/mm^3)	2995 ± 134	2543 ± 78**	2637 ± 68	2129 ± 75***
Mean "true" capillary cross-sectional area (μm^2)	28.3 ± 1.0	26.9 ± 1.3	23.6 ± 0.9	24.4 ± 0.4
Mean "true" myocardial cell cross-sectional area (μm^2)	291 ± 13	340 ± 10**	343 ± 8	432 ± 15***
3-dimensional capillary-fiber ratio	1.26 ± 0.04	1.50 ± 0.04***	1.41 ± 0.03	1.43 ± 0.07
3. ULTRASTRUCTURE OF THE MYOCARDIAL CELL				
Volume of myofibrils/myocardial cell cytoplasmic volume (%)	64.8 ± 1.9	65.2 ± 1.0	74.2 ± 0.6	73.6 ± 0.7
Volume of mitochondria/myocardial cell cytoplasmic volume (%)	26.5 ± 1.1	27.3 ± 1.0	23.7 ± 0.6	22.9 ± 0.7

Abbreviations: C/EX = exercise model, control group; H/EX = exercise model, hypertrophy group; C/PO = pressure overload model, control group; H/PO = pressure overload model, hypertrophy group. Results are given as arithmetic means ± SEM. The means were compared by Student's t-test between groups within models. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

DISCUSSION

In previous morphometric studies the analysis of capillary vascularization was most often restricted to counts and measurements of planar capillary profiles in transverse sections. Thus "capillary density", the number of capillary profiles per tissue area in transverse sections, has been adopted as the key descriptor of myocardial capillarization. However, capillary density as observed on sections is influenced by capillary length density and the degree of anisotropy, which in turn depends partially on the state of muscular contraction; thus it is in fact, despite its easy accessibility, the multivariate result of a complex 3D geometrical reality. Therefore "capillary density" is inferior to capillary length density as a descriptor of capillarization. Clearly this statement is but one example for the fact that stereological estimates are of higher conceptual purity, and thus biological significance, than are their 2D counterparts which look often deceivingly simple at first glance. Accordingly, the stereological parameters: mean "true" cross-sectional area, 3D capillary-fiber ratio, and anisotropy constant of capillary axis orientation distribution were introduced by us into quantitative myocardial microscopy to investigate the capillary geometry in full depth. In contrast, correction for partial anisotropy was found to be negligible for myocardial cells in the left ventricular papillary muscles.

The central result is a different reaction pattern of the capillaries in our 2 models of mild cardiac hypertrophy. There is compensatory capillary proliferation in exercise-induced hypertrophy which leads to an increased 3D capillary-fiber ratio, thus supplying the thickened myocardial cell with more capillaries and keeping mean oxygen diffusion distance constant. Pressure overload induced hypertrophy lacks this compensatory mechanism. The hypothesis is now well founded that increased myocardial blood flow can evoke capillary proliferation. Each acute exercise bout leads to a temporary increase of myocardial perfusion, whereas pressure overload cannot be expected to elicit increased myocardial blood flow. Hence the observed differences between the stereological reaction patterns may well be explained on the ground of different blood flow in the capillary bed.

ACKNOWLEDGMENT
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