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Effect of ageing and malnutrition on rat myocardium

II. The microvasculature

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Summary. The modulating effects of ageing and malnutrition on rat myocardium were studied morphometrically with respect to the microvasculature. An increase in capillary density together with a decrease in capillary lumen cross-sectional area was noted during starvation. The important changes seen in the myocyte T-system were paralleled by a decreased diffusion distance for oxygen from the capillary lumen to the mitochondrion. The changes described in the aged rat heart point to an altered inter-relationship between parenchyma and vascularization with a lower capillary volume fraction and a greater diffusion distance from the capillary lumen to the mitochondrion; this is caused by hypertrophy of the aged myocyte. This reduction in capacity to exchange substrates is further reduced by the less developed T-system in the older myocyte.

Key words: Malnutrition – Ageing – Myocardium – Microvasculature – Morphometry

Introduction

The inter-relationship of the parenchymal cell population and the microvasculature is critical in normal organ function. Since nutrition and ageing have specific and opposing effects on the cellular and parenchymal component of the myocardium with regard to exchange capacity (Vandewoude and Buyssens 1992) it is important to examine the changes induced on the microvasculature under experimental conditions of malnutrition and ageing. Quantitative estimation of the volume fractions of the cellular compartments relates to the functional capacity of the tissues (Schaper et al. 1985). Interactions between microvascular and cell compartment can be characterized by three fundamental variables: (1) capillary luminal volume density, (2) capillary luminal surface density, and (3) the average maximum diffusion distance for oxygen (Weibel 1979; Hoppeler et al. 1981; Anversa et al. 1984). Volume density relates to the volume of capillary blood available for gas exchange within the tissue; surface density relates to the area available for metabolic interchange of oxygen and metabolites; and diffusion distance describes the distance oxygen travels between the capillary and the mitochondria, where oxygen is consumed in generating ATP through oxidative phosphorylation.

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Materials and methods

Perfusion-fixation of rat myocardium was performed in groups of 16-week-old, female Wistar rats ($n=5$–7) during starvation at days 0, 1, 2, 4, 6, 8 and 10, in 16-week-old control rats and in a group of 24-month-old rats ($n=6$) of the same strain. The animal model, the animal fixation procedure and morphometric techniques for evaluating volume fractions and surface densities were as previously described (Vandewoude and Buyssens 1992). The numbers of profiles of myocytes and capillaries per unit area of tissue cross-section were counted and the capillary myocyte ratio was calculated (Angelakos et al. 1964; Aherne and Dunnill 1982). The maximum diffusion distance for oxygen can be measured by using the Krogh's cylinder model for gas exchange in tissue (Weibel 1979). This assumes that capillaries are uniformly distributed in the myocardium and that the mitochondria are dispersed evenly throughout the myocyte cytoplasm. The maximum distance from the capillary wall to the mitochondria of myocytes (R) can then be calculated from the capillary profile density, in transverse myocardial sections according to the equation:

$$R=\sqrt{\frac{1}{\pi \cdot N_A}}-\sqrt{\frac{\bar{A}}{\pi}}$$

where $\bar{A}$ is the average cross-sectional area of capillary lumen (measured by planimetry) and N_A the number of capillaries per unit area of myocardium (Anversa et al. 1984).

Results

A decrease in capillary volume fraction was noted with ageing as determined by point counting on low-power

Table 1. Volume fractions of capillaries (endothelium and luminal surface), vascular endothelial cells and intercellular matrix (IM) in rat heart in controls, after starvation and in aged rats

	Capillary	Vascular endothelium	IM
Control			
LV	15.6±2.0	4.8±0.8	4.1±1.0
RV	15.8±2.1	5.2±0.6	2.0±0.3
Fasted			
LV	15.2±2.2	3.4±0.4	3.3±0.8
RV	15.3±1.7	4.7±0.7	3.5±0.4**
Aged			
LV	11.5±1.0**	3.2±0.3	3.1±0.8
RV	8.5±0.7*,**	2.5±0.4***	3.8±0.3***

Data expressed as percentage volume fraction (mean±standard error). LV, Left ventricle; RV, right ventricle

* $P<0.05$: compared with LV
** $P<0.01$: compared with controls
*** $P<0.005$: compared with controls

Table 2. Surface density (μ^{-1}) of capillaries and myocytes in left (LV) and right (RV) rat heart ventricle in controls compared to 10-day fasted and aged rats

	Control	Fasted	Aged
Left ventricle			
Capillaries	0.96±0.08	0.87±0.05	1.36±0.14*
Myocytes	0.27±0.03	0.43±0.04**	0.27±0.03
Right ventricle			
Capillaries	1.02±0.10	0.97±0.06	1.16±0.10
Myocytes	0.37±0.03	0.41±0.03	0.26±0.03*

Data expressed as mean±standard error

* $P<0.05$
** $P<0.01$

electron micrographs (×3340) (Table 1). The reduction in lumen was equal to that in vascular endothelium. A slight increase was seen in the intercellular matrix compartment both during fasting and ageing.

Capillary surface density in the left or right ventricle did not change significantly during fasting, but did tend to increase during ageing (Table 2).

Figure 1 depicts the evolution of the maximum diffusion distance for oxygen in the heart ventricles during acute starvation. In the left ventricle, a progressive shortening of the distance was observed; in the right ventricle, no significant change was observed. Tables 3 and 4 show the myocardial capillary characteristics in controls, after starvation, and after ageing.

Capillary volume fraction did not change with starvation. There was a decrease in lumen cross-sectional area but also a significantly smaller maximal diffusion capacity in the left ventricle in the malnourished rat. The relation between capillaries and myocytes did not change as indicated by the constant capillary/myocyte ratio. The

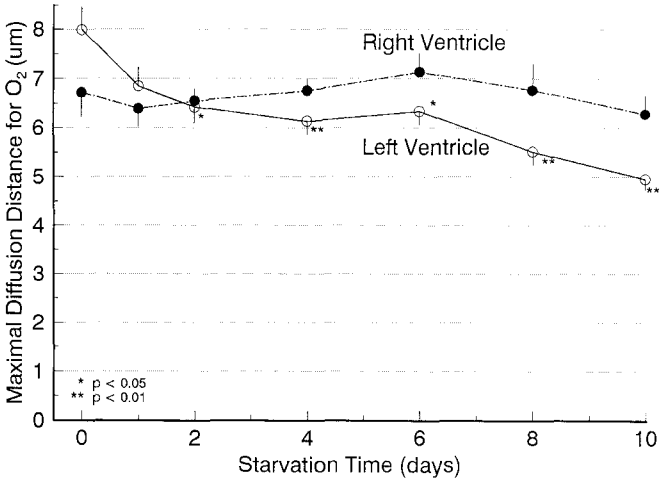

Fig. 1. Maximal diffusion distance for oxygen in myocytes from the rat left and right heart ventricle during total starvation

available surface area per unit volume for metabolic interchange remains the same. In older rats the capillary volume fraction was lower but surface density increased. The maximum diffusion distance was also greater in the aged rat.

Discussion

The alterations and modulations induced by ageing and by total starvation (as a model of pure protein-calorie malnutrition) were analysed by measuring structural variables in the capillary bed, which regulates tissue oxygen and substrate distribution and consumption.

An increase in capillary density is noted, together with a decrease in capillary lumen cross-sectional area, during starvation. The ratio of the number of capillaries to myocytes does not change nor do volume fraction and surface density of the capillaries. The maximum diffusion distance for metabolites decreases significantly, which improves the capacity of the malnourished heart to interchange oxygen and metabolites with the plasma compartment. This can be a major factor in preservation of ventricular function, despite marked morphological alterations (Drott et al. 1986; Sjostrom et al. 1987). The extent of these changes is closely comparable to that described by Frenzel et al. (1988) in their studies of the regression of exercise-induced cardiac hypertrophy.

The situation is totally different in the older rat heart where the capillary volume fraction is reduced, the maximum diffusion distance is longer, and the capillary surface density is higher. The overall effect is a less favourable situation regarding metabolic substrate interchange (Ricci et al.1987).

Morphometric data on the myocardium in the literature deal mainly with cellular changes caused by hypertension, aortic constriction or increased load on the myocardium by exercise. Engelmann et al. (1987) observed a drastic decline in capillary density in the left ventricle of spontaneously hypertensive rats (SHR). He showed that young SHR had the same low capillary

Table 3. Capillary characteristics in left heart ventricle in normal control rats, malnourished rats, and older rats

	Control	Starved	Older
V_v Capillaries (%)	15.6 ±2.0	15.2 ±2.2	11.5 ±1.0*
Lumen	10.8 ±0.8	11.8 ±0.4	8.3 ±0.3
Endothelium	4.8 ±0.8	3.4 ±0.4	3.2 ±0.3
S_v Capillaries (μ^{-1})	0.96 ±0.08	0.87 ±0.05	1.36 ±0.14*
CSA Capillaries (μ^2)	30.5 ±1.9	21.3 ±1.2*	33.6 ±2.8
C/M ratio	0.945±0.047	0.939±0.082	1.005±0.064
Max. diffusion distance (μ)	7.99 ±0.48	4.96 ±0.21**	8.87 ±0.72

Data expressed as mean±standard error. V_v, Volume fraction per unit myocardium; S_v, surface density; CSA, cross-sectional area; C/M, ratio of capillary to myocyte profiles.

* $P<0.05$: compared to control group
** $P<0.01$: compared to control group

Table 4. Capillary characteristics in right heart ventricle in normal control rats, malnourished rats, and older rats

	Control	Starved	Older
V_v Capillaries (%)	15.8 ±2.1	15.3 ±1.7	8.5 ±0.7*
Lumen	10.6 ±0.6	10.6 ±0.7	6.0 ±0.4**
Endothelium	5.2 ±0.6	4.7 ±0.7	2.5 ±0.4**
S_v Capillaries (μ^{-1})	1.02 ±0.10	0.97 ±0.06	1.16 ±0.10
CSA Capillaries (μ^2)	27.9 ±1.9	21.3 ±1.3	30.7 ±2.9
C/M ratio	0.951±0.082	0.964±0.049	1.010±0.072
Max. diffusion distance (μ)	6.72 ±0.50	6.29 ±0.28	11.2 ±1.04*

Data are expressed as mean±standard error. V_v, Volume fraction per unit myocardium; S_v, surface density; CSA, cross-sectional area; C/M, ratio of capillary to myocyte profiles

* $P<0.05$: compared to control group
** $P<0.01$: compared to control group

density as senescent rats. In hypertension there is an increase in myocyte volume with a higher myofibrillar-mitochondrial ratio and an increase in sarcoplasmic reticulum and T-system volume and surface (Anversa et al. 1978; Lund and Tomanek 1978; Bishop et al. 1979). Some authors have found an unchanged myofibrillar mitochondrial ratio (Breisch et al. 1980; Anversa et al. 1983) or even a reduced ratio in exercise-induced hypertrophy (Mattfeldt et al. 1986; Frenzel et al. 1988).

In hypertrophy induced by hypertension (Anversa et al. 1986; Engelmann et al. 1987) or exercise (Loud et al. 1984; Frenzel et al. 1988) a deficit in capillary luminal surface density and capillary volume density was noted together with an increase in maximum diffusion distance for oxygen. The regression of exercise-induced (Frenzel et al. 1988) or hypertension-induced (Breisch et al. 1980) hypertrophy resulted in almost complete reversal of the changes induced by hypertrophy.

The reduction in the size of myocytes was accompanied by a quantitative normalization of most of the cellular organelles. The observed cellular and ultrastructural alterations with ageing in our study also showed a hypertrophy of the myocytes with a lower myofibrillar mitochondrial ratio (Vandewoude et al. 1992), a reduction in capillary volume fraction and a longer maximum diffusion distance for oxygen. These changes are very much the same as those induced by hypertrophy, especially exercise-induced hypertrophy. With hypertension-induced hypertrophy the volume fraction of mitochondria is better preserved but in every type of hypertrophy there seems to be a certain degree of vascular insufficiency.

In malnutrition-induced atrophy, however, the capacity of the myocyte for metabolic exchange with its direct environment seems to be improved due to the higher surface per unit volume available. This phenomenon is seen at the level of the myocyte itself and its cell organelles. This creates a more favourable situation for the starved myocytes. It is possible that this atrophy at the cellular level is one of the mechanisms that increase longevity in rodents on caloric restriction (Masoro 1987), by virtue of improved capillary exchange. The opposite is true in ageing, where hypertrophy at the cellular level leads progressively to deterioration of vascularization of the myocardium. Hypertrophy at younger age induced by a pathological state such as hypertension will only accelerate this process.

We conclude that the atrophic alterations at the cellular level in rat myocardium during starvation are different, and contrast with those induced by ageing. Physiological deterioration with ageing is associated with a situation of vascular deficiency that ultimately leads to tissue ischaemia and cell necrosis. The morphological changes which accompany the regression during malnutrition protect the starved myocyte by increasing its potential for metabolic exchange.

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