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Synergistic effects of diabetes mellitus and renovascular hypertension on the rat heart – stereological investigations on papillary muscles*

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Summary. The effects of combined renovascular hypertension and diabetes mellitus on the rat heart were investigated in order to detect possible synergistic effects of the two conditions. Hypertensive diabetic and hypertensive nondiabetic young male Wistar rats were compared with diabetic and nondiabetic controls. Since the normal body weight increase of the diabetic animals was markedly suppressed a weight-matched nondiabetic control group was introduced in addition. Hypertension was established for eight weeks by a surgical stenosis of the left renal artery, diabetes mellitus was maintained for four weeks after a single intraperitoneal injection of 75 mg/kg streptozotocin. Light and electron microscopic stereological parameters were obtained for the left ventricular papillary muscles. The whole hearts were also investigated histologically. Qualitative morphology failed to substantiate synergistic effects in the hypertensive diabetic rats. Vascular abnormalities were not observed. The stereological parameters, however, revealed microstructural reactions which were observed exclusively in the hypertensive diabetic group: the volume ratio of mitochondria-to-myofibrils was decreased, the surface-to-volume ratio of mitochondria was increased (reduction of mitochondrial size) and the mean cross sectional area of capillaries was decreased. Similar quantitative mitochondrial changes have been frequently described in long-standing hypertension, but in the present investigation, they were not found in the nondiabetic hypertensive group. It is therefore concluded that diabetes mellitus potentiates the effects of chronic pressure overload on myocardial cells. However, the myocardial fibrosis which has been found by other groups at later stages of hypertension and/or diabetes mellitus was not detected in the present study. The reduced mean cross sectional area of capillaries in hypertensive-diabetic rats may be correlated with early molecular changes of the myocardial interstitium or with early abnormalities of small arteries. Thus our stereological results support the hypothesis that a non-coronary hypertensive diabetic cardiomyopathy occurs in mammalian hearts.

Key words: Hypertension – Diabetes mellitus – Cardiac hypertrophy – Myocardial ultrastructure – Morphometry

Introduction

Hypertension and diabetes mellitus are frequent disorders in modern industrial countries. Epidemiological studies provide evidence that both conditions are important risk factors for coronary heart disease and congestive heart failure. Cardiac damage, however, may occur even in the absence of significant atherosclerosis of the coronary arteries (McKee et al. 1971; Kannel et al. 1974).

Recently it has been shown that the coexistence of diabetes mellitus and hypertension may combine to enhance myocardial alterations. Combination of hypertension and diabetes mellitus was associated with a more pronounced myocytolysis and myocardial fibrosis when compared with hypertension and diabetes alone. The existence of a hypertensive diabetic cardiomyopathy has been postulated on the base of autopsy studies on human hearts (Factor et al. 1980) and by experimental

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** Dedicated to Prof. Dr. Drs.h.c.mult. G. Schettler on the occasion of his 70th birthday

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models of combined renovascular hypertension and diabetes mellitus induced by streptozotocin (Factor et al. 1981). In hypertensive diabetic rats, congestive heart failure may develop after some months (Fein et al. 1984).

Qualitative ultrastructural investigations on hearts of hypertensive diabetic rats revealed potentiation of damage to myocardial cells (Factor et al. 1983). This was substantiated by qualitative evaluation of electron microscopic images. It seems to be useful, however, to analyse the pathogenesis of hypertensive diabetic cardiomyopathy on the basis of more precise data on the myocardial structure. In the present study, modern techniques of tissue fixation and quantitative stereology were employed in order to obtain objective and reliable information on the quantitative structural changes in the myocardium.

Materials and methods

Eighty young male Wistar rats (body weights: 130–150 g, Ivanovas, Kisslegg, FRG) were divided into four groups by the use of random numbers. All animals were caged individually and received chow pellets and tap-water ad libitum.

Twenty animals were subjected to moderate renovascular hypertension for eight weeks. Stenosis of the left renal artery was induced surgically with a silver clip of 0.20–0.22 mm internal diameter. After induction of hypertension, a 2% NaCl solution was offered in order to prevent the development of malignant hypertension (Möhring et al. 1976).

A further twenty five animals were also subjected to moderate renovascular hypertension for eight weeks. After four weeks, diabetes mellitus was induced with a single intraperitoneal injection of 75 mg/kg body weight streptozotocin.

Twenty animals were sham-operated and were treated with streptozotocin (75 mg/kg body weight) after four weeks, as a diabetic group.

Fifteen sham-operated animals served as controls.

In young diabetic rats, the normal increase in body weight is considerably suppressed. A weight-matched control group with similar body weights when compared with the diabetic rats was therefore included in the present investigation ($n=7$).

Systolic blood pressure was determined by means of a tail-plethysmographic method in slight ether anesthesia after three, four, six and eight weeks. On each occasion two blood pressure recordings were performed in order to test the reproducibility of the measurements. Operated animals with a systolic blood pressure lower than than 140 mmHg at any time were excluded from the study. Streptozotocin-injected animals were investigated with glucose oxidase kits (Boehringer Mannheim, FRG). Streptozotocin-injected animals which had blood glucose levels lower than 11.0 mmol/l were excluded from the study.

After eight weeks, the viscera were fixed by retrograde vascular perfusion at a pressure of 110 mmHG after catheterization of the abdominal aorta as described elsewhere (Mall et al. 1987). Left ventricular papillary muscles were cut randomly either longitudinally or transversely with a tissue sectioner as described elsewhere (Mattfeldt and Mall 1984; Mall et al. 1978). Seven transversely cut 200 µm slices and two longitudinally cut 200 µm slices were randomly selected for stereology and embedded in Epon-Araldite as described elsewhere (Mall et al. 1986a; Mall et al. 1987). Semithin sections (1 µm) were stained with methylene blue and basic fuchsin (Di'Sant'Agnese and De Mesy Jensen 1984) and examined by light microscopy using oil immersion and phase contrast. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope.

In addition, transverse slices of the total heart were embedded in Paraplast, sectioned at 3–4 µm, and stained with haematoxylin-eosin and the Masson-Goldner trichrome stain.

Quantitative stereology. Myocardial tissue is composed of anisotropic structure components of which the directions in space are concentrated around the longitudinal axis of the muscle with variable degrees of concentration. In stereological terminology, the longitudinal axis is designated as the axis of anisotropy. If papillary muscles are investigated, the longitudinal axis can be detected macroscopically.

Estimation of volume densities (V_V: volume per unit reference volume) is not influenced by anisotropic features of the tissue. The V_V's were obtained by point counting (Weibel 1979) using the standard equation.

$$V_V = P_P \tag{1}$$

In contrast, estimation of length densities (L_V: length per unit reference volume) and surface densities (S_V: surface area per unit reference volume) depends on the orientation of the structural elements in space and the angle α between the section plane and axis of anisotropy. Recently, we have introduced stereological procedures to obtain L_V and S_V of capillaries and S_V of mitochondria which are based on mathematical models of directional statistics (Weibel 1980; Mattfeldt and Mall 1984; Mall et al. 1986a; Mattfeldt et al. 1986; Mall et al. 1987).

Length densities (L_V) and surface densities (S_V) were calculated according to the following stereological equations (Weibel 1980; Mattfeldt and Mall 1984; Mall et al. 1986):

$$L_V = c_1(K_L,\ \alpha=0) * Q_A(\alpha=0) \tag{2}$$
(capillaries, myocardial cells)

$$S_V = c_2(K_S,\ \alpha=0) * B_A(\alpha=0) \text{ (capillaries)} \tag{3}$$

$$S_V = (4/3\pi) * \{B_A(\alpha=0) + 2\ B_A(\alpha=\pi/2)\} \tag{4}$$
(surface of mitochondria)

The $\{Q_A(\alpha=0)\}$'s are the commonly used planar parameters: density of myocardial cells and capillaries in transverse sections, e.g. number of profiles per unit transverse sectional area. They are to be multiplied with the correction factor $c_1(K_L, \alpha=0)$. Analogously, the boundary length of capillary profiles per unit transverse sectional area ($B_A(\alpha=0)$ is to be multiplied with the correction factor $c_2(K_S, \alpha=0)$.

The degree of anisotropy, e.g. concentration around the axis is quantified by the constants of anisotropy K_L and K_S. They are derived from the ratio of counts on transverse ($\alpha=0$) and longitudinal sections ($\alpha=\pi/2$): $Q_A(\alpha=0)/Q_A(\alpha=\pi/2)$ and $B_A(\alpha=0)/B_A(\alpha=\pi/2)$, and the correction coefficients $c_1(K_L, \alpha=0)$ and $c_2(K_S, \alpha=0)$ are calculated as described elsewhere (Mattfeldt and Mall 1984).

From equation (4), we obtain S_V estimation provided that the degree of anisotropy is low $\{B_A(\alpha=0);/B_A(\alpha=\pi/2) \leq 6/5\}$ (Weibel 1980; Mall et al. 1986a).

The stereological analysis was performed on transverse and longitudinal sections of the left ventricular papillary muscles as a multistage sampling procedure. In the first stage (magnification 1000:1, light microscopy) seven transverse and two longitudinal semithin sections per animal were evaluated. Eight test areas per section (58000 µm^2) were analysed with a Zeiss eyepiece containing 100 points and 10 parallel lines (total

length: 927 μm). Test areas were selected by random systematic subsampling. The points were used for V_V estimation and B_A was estimated by counting the intersections of the capillary profile boundaries with the test lines. V_V of total interstitial tissue, V_V of myocardial cells and V_V of capillary lumina were obtained from transverse sections, and L_V and S_V of capillaries from transverse and longitudinal sections. Reference volume was the total myocardial tissue of the left ventricular papillary muscles.

In the second stage (magnification 4000:1, electron microscopy): Two transverse ultrathin sections per animal were randomly selected. 10 random test areas per section were systematically subsampled. The length density (L_V) of myocardial cells per unit test area ($Q_A(0)$) was determined at this stage. Previous studies (Mattfeldt et al. 1986) have shown that $Q_A(0)$ of myocardial cells is a nearly unbiased estimate of L_V of myocardial cells in papillary muscles, since $Q_A(\pi/2)$ tends to 0 and the correction coefficient $c_1(K_L, 0)$ to 1. In other words, the axes of myocardial cells are oriented in parallel (Mattfeldt et al. 1986).

In stage 3 (magnification 32500:1, electron microscopy) two randomly selected transverse ultrathin sections per animal were used to estimate V_V of capillaries and V_V of (non-capillary) interstitial tissue. These sections were supported on nickel grids containing 300 meshs. 15 meshes per section were systematically subsampled. The total area of each mesh was divided into 16 areas each containing 80 test points. The volume ratios capillaries per total interstitial tissue and noncapillary interstitium per total interstitial tissue were estimated with point counting. Sampling and counting was performed on-line on a television monitor. The ratio estimates were multiplied with V_V of total interstitial tissue obtained at stage 1. Thus, the reference volume was the total myocardial tissue of left ventricular papillary muscles (Mall et al. 1987).

In addition, ten random test areas with 80 test points per area were obtained by systematic subsampling (distance between 2 points: 0.5 μm), to determine V_V mitochondria, V_V myofibrils and V_V sarcoplasmic matrix. 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). The reference volumes were the myocardial cells of the left ventricular papillary muscles (Mall et al. 1986a).

From L_V of capillaries and L_V of myocardial cells, the "true" three-dimensional capillary-fiber ratio, e.g. the length of capillaries (mm) per unit length of muscle cells (mm), was derived by division (L_V of capillaries/L_V of myocardial cells). The latter is a more realistic parameter of capillarization than the planar ratio Q_A of capillaries divided by Q_A of myocardial cells (=capillary profiles per myocardial cell profiles in transverse sections) hitherto used (Mattfeldt et al. 1986; Mall et al. 1987).

From L_V and V_V of myocardial cells and capillaries, respectively, the "true" mean cross sectional areas a were determined according to the equation: $a = V_V/L_V$. Note that a is the mean of all profile sectional areas normal to the axes and not the mean of all sectional areas in random transverse sections (Mathieu et al. 1983; Mattfeldt et al. 1986).

One-way analysis of variance was used to compare arithmetic means between the five groups. A result was considered to be significant it the probability of error, p, was lower than 0.05. Divergences between two groups were tested according to Scheffe (Sachs 1974). A result was considered to be significant if the probability of error, p, was lower than 0.05.

Results

In all treated groups some animals died during the experimental period; 5/18 animals in the hypertensive group, 4/15 animals in the diabetic group and 11/22 animals in the hypertensive diabetic group. Histological examination revealed the absence of myocarditis or pneumonia. Congestive heart failure was not observed in these animals.

The systolic blood pressure was increased to 172 ± 21 mmHg in the hypertensive group as compared to 91 ± 5 mmHg in the control group ($+89\%$, $p < 0.001$, Student's t test) and to 151 ± 23 mmHg in the hypertensive diabetic group as compared to 79 ± 7 mmHg in the diabetic group ($+91\%$, $p < 0.001$, Student's t test).

The blood glucose levels were increased to 16.2 ± 1.9 mmol/l in the diabetic group as compared to 5.1 ± 0.3 mmol/l in the control group ($+318\%$, $p < 0.001$, Student's t test) and to 16.9 ± 2.9 mmol/l in the hypertensive diabetic group as compared to 5.5 ± 0.3 mmol/l in the hypertensive group ($+307\%$, $p < 0.001$, Student's t test).

Table 1 shows that the normal growth of the animals was considerably suppressed in both diabetic groups which is indicated by the lower body and heart weights, respectively. The heart-to-body weight ratio in the diabetic group, however, was not changed when compared with the two control groups (Tables 1, 2). Heart weight increase was

Table 1. Heart weights and body weights

Parameter	Control group $n=12$	Hypertensive group $n=12$	Weight-matched control group $n=7$	Diabetic group $n=10$	Hypert. diabetic group $n=11$	Level of statist. signific.
Body weight (g)	287 ±26	267 ±50	175 ±7	178 ±15	159 ±18	$p<0.001$
Heart weight (g)	0.884± 0.098	1.171± 0.137	0.590±0.084	0.578± 0.056	0.753± 0.094	$p<0.001$
Heart/body weight (mg/g)	3.09 ± 0.30	4.47 ± 0.61	3.37 ±0.37	3.25 ± 0.26	4.78 ± 0.69	$p<0.001$
LVW/body weight (mg/g)	2.56 ± 0.24	3.78 ± 0.56	2.74 ±0.25	2.63 ± 0.26	3.99 ± 0.64	$p<0.001$

Means ± standard deviations; LVW: Left ventricular weight

The means of the five groups were tested by one way analysis of variance. A result was considered to be significant if $p < 0.05$

Table 2. Significant divergences between the groups Body weights and heart weights (Scheffe's Test)

Parameter	C_1–H	C_1–C_2	C_2–D	C_2–HD	D–HD
Body weight	N.S.	$\downarrow p<0.001$	N.S.	N.S.	N.S.
Heart weight	$\uparrow p<0.001$	$\downarrow p<0.001$	N.S.	$\uparrow p<0.001$	$\uparrow p<0.001$
Heart/body weight	$\uparrow p<0.001$	N.S.	N.S.	$\uparrow p<0.001$	$\uparrow p<0.001$
LVW/body weight	$\uparrow p<0.001$	N.S.	N.S.	$\uparrow p<0.001$	$\uparrow p<0.001$

↓: Significant decrease; ↑: Significant increase; N.S.: $p \geq 0.05$; LVW: Left ventricular weight; C_1: Control group; H: Hypertensive group; C_2: Weight-matched control group; D: Diabetic group; HD: Hypertensive diabetic group

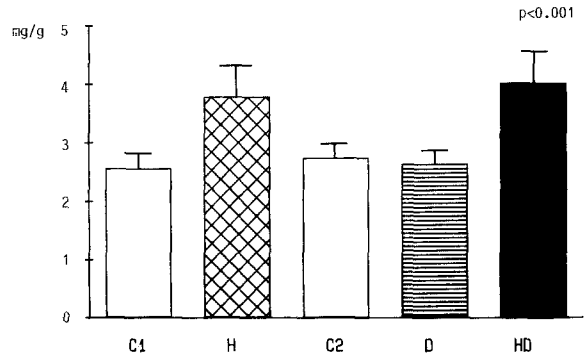

Fig. 1. Relative left ventricular weight (ratio of left ventricular weight to body weight) is increased in hypertensive diabetics and hypertensive nondiabetics at a comparable degree. Note that the ratios are not different between the two control groups and the diabetic group. C_1: Control group; C_2: Weight-matched control group; H: Hypertensive group; D: Diabetic group; HD: Hypertensive diabetic group

found in the hypertensive and the hypertensive-diabetic group to a comparable degree when compared with the control group and the weight-matched control group respectively (+32% and 28%, $p<0.001$, Table 2). The similar degree of hypertrophy in diabetic and non-diabetic animals is clearly demonstrated in Fig. 1 by the relative left ventricular weights.

Qualitative histological investigation of transverse sections of the whole hearts revealed the absence of significant fibrotic changes in all groups. Qualitative changes of the intramural vessels were not observed. Electron microscopic evaluation of the left ventricular papillary muscles showed a normal ultrastructure in the hypertensive group (Fig. 2). A slight increase in sarcoplasm including an increase in beta-glycogen granules was regularily found in both the diabetic and the hypertensive-diabetic group (Figs. 3, 4). Significant structural damage of mitochondria and myofibrils, however, could not be detected. The ultrastructural components of the myocardial interstitium, especially the basement membranes of capillaries, were not significantly altered (Fig. 5).

The quantitative stereological parameters (Tables 3–5) established the following effects of the different treatments:

1. The mean cross sectional areas (MCSA) of myocardial cells in left ventricular papillary muscles are significantly different between the five groups ($p<0.001$, Table 3). They are obviously associated with the different heart weights {(HW) (Table 1)} and linear regression analysis which was applied to logarithmically transformed data yields

$$\mathrm{MCSA} = 365.8 * \mathrm{HW}^{0.75} \quad (5)$$

Interestingly, curve fitting between the left ventricular muscle mass {LMM = left ventricular weight × volume density of myocardial cells (calculated from Tables 1, 3, 4)} and MCSA corresponds to harmonic growth of muscle fibers {LMM ≈ (MCSA * total fiber length)} where MCSA ~ $\mathrm{LMM}^{2/3}$ and total fiber length ~ $\mathrm{LMM}^{1/3}$:

$$\mathrm{MCSA} = 450.7 * \mathrm{LMM}^{0.68} \quad (6)$$

Note that equation (6) is derived from morphometric parameters which are obtained from left ventricular papillary muscles (Fig. 6) and not from the whole left ventricle.

2. The length densities (L_V) and surface densities (S_V), of capillaries and the volume density (V_V) of capillary lumina are significantly different between the five groups ($p<0.001$). In contrast, the three-dimensional capillary-fiber ratios did not reveal significant divergences. Thus the difference in capillary density parameters should be inversely proportional to the MCSA ($\sim \mathrm{MCSA}^{-1}$). From the data in Table 3 and the MCSA's we obtain:

$$L_V(\mathrm{cap}) = 820.0 * \mathrm{MCSA}^{-0.93} \quad (7)$$

$$S_V(\mathrm{cap}) = 113.3 * \mathrm{MCSA}^{-0.89} \quad (8)$$

$$V_V(\mathrm{cap}) = 10.04 * \mathrm{MCSA}^{-0.83} \quad (9)$$

The lower absolute value of the exponent in equation (9) as compared to equation (7) is to be related to effects of combined diabetes and hypertension

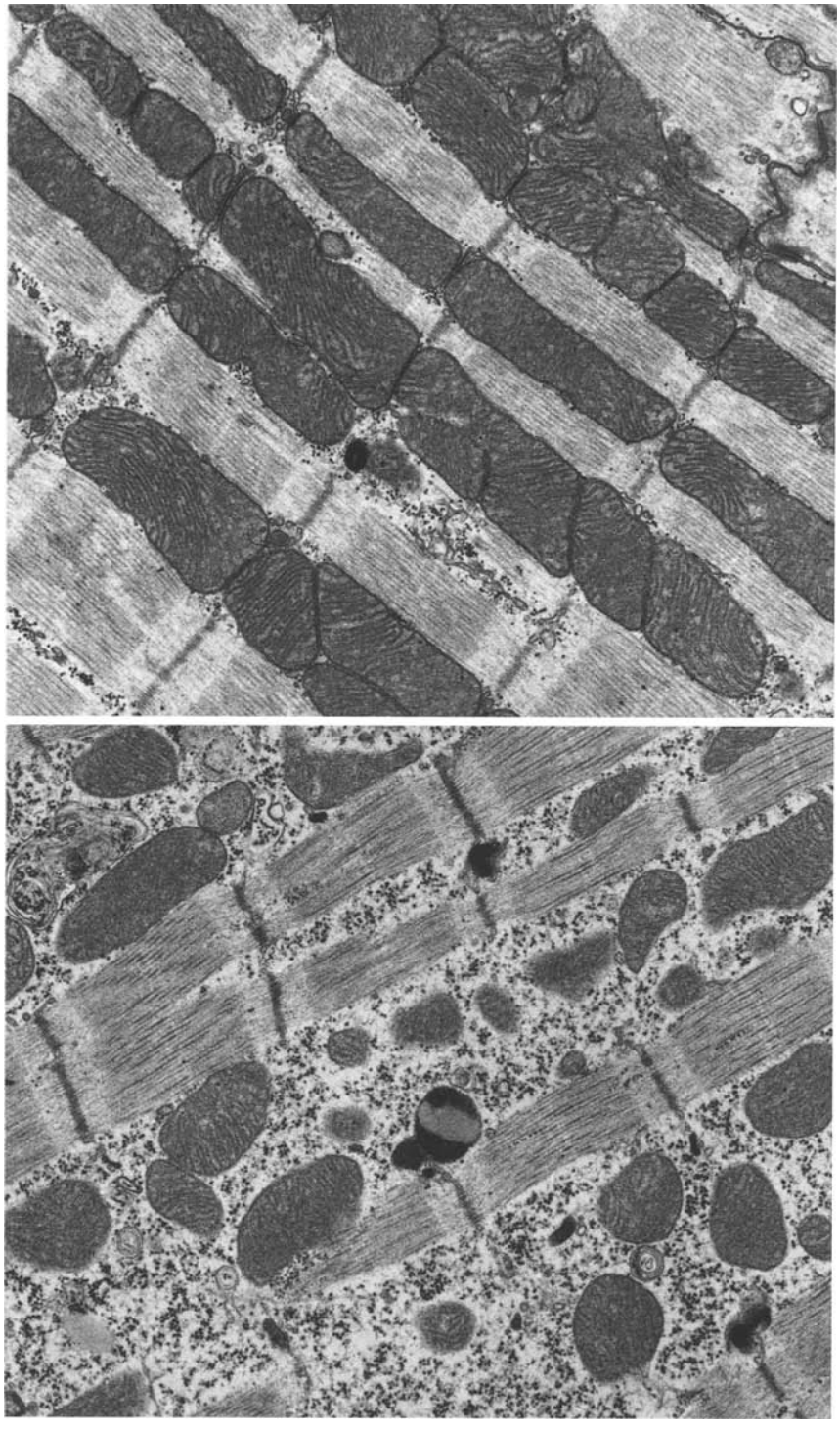

Fig. 2. Myocardial cell ultrastructure of a hypertensive nondiabetic rat without significant alterations (electron micrograph, magnification ×22100)

Fig. 3. Sarcoplasmic oedema and increase in beta-glycogen granules in diabetic rats. Mitochondrial abnormalities are not observed (electron micrograph, magnification ×20100)

on capillaries. This is substantiated by the lower capillary mean cross sectional areas in the hypertensive diabetic group as compared to the other groups ($p < 0.001$; Fig. 7, Table 3).

3. The volume density of non-capillary interstitial cells, e.g. pericytes and fibroblasts, is moderately increased in both hypertensive groups without any convincing evidence for a potentiation induced by diabetes. However, the volume density of the non-cellular interstitial matrix including collagen was not significantly affected by the different treatments (Table 4).

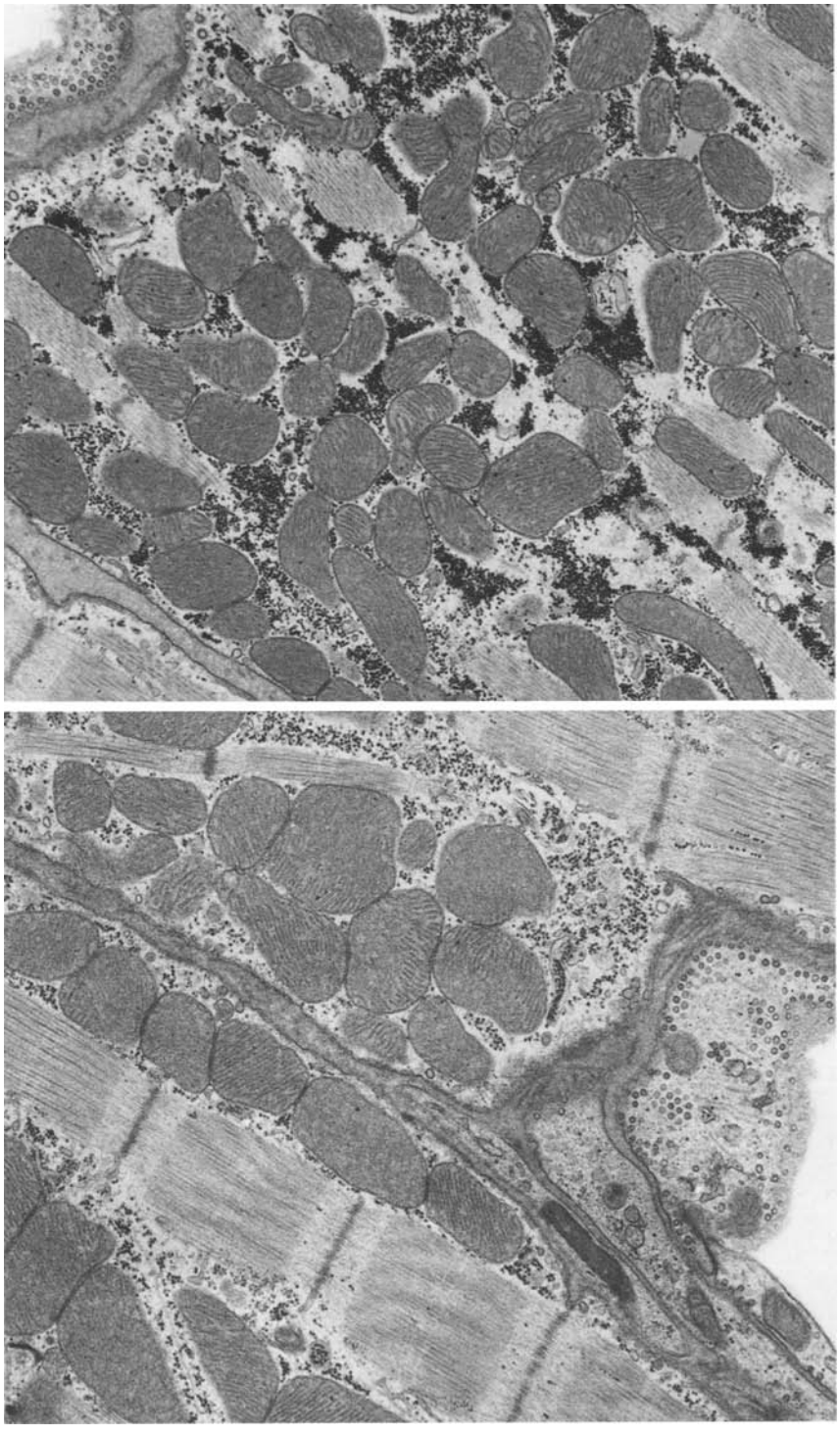

Fig. 4. Sarcoplasmic oedema and beta-glycogen granules in hypertensive diabetic rats. Note that oedema is not increased by hypertension (electron micrograph, magnification × 19300)

Fig. 5. Normal ultrastructure of the myocardial interstitium in hypertensive diabetic rats. Alterations of capillary basement membranes are not detected (electron micrograph, magnification × 19500)

4. The stereological parameters of myocardial cell organelles resemble the following reactions induced by the different treatments: a) Diabetes mellitus increases the volume density of the sarcoplasm in both the diabetic and the hypertensive diabetic group (Tables 6, 7). b) The volume-to-volume ratio: mitochondria-to-myofibrils is considerably decreased in the hypertensive diabetic group as compared to the other groups (Fig. 8, Tables 6, 7). Note that V_V of mitochondria and V_V of myofi-

Table 3. Length densities (L_V), surface densities (S_V), and mean cross sectional areas of capillaries and myocardial cells

Parameter	Control group $n=12$	Hypertensive group $n=12$	Weight-matched control group $n=7$	Diabetic group $n=10$	Hypert. diabetic group $n=11$	Level of statist. signific.
Capillaries						
L_V (mm/mm^3)	3718 ±237	2912 ±284	4549 ±324	5065 ±365	4257 ±509	$p<0.001$
$Q_A(\alpha=0)/Q_A(\alpha=\pi/2)$	5.5 ± 0.4	4.6 ± 0.8	5.5 ± 0.8	5.4 ± 0.6	5.4 ± 0.7	$p<0.05$
Constant of anisotropy (K_L)	5.8	4.4	5.8	5.7	5.7	
S_V (cm^2/cm^3)	673 ± 51	552 ± 71	822 ± 58	878 ± 70	688 ± 96	N.S.
$B_A(\alpha=0)/B_A(\alpha=\pi/2)$	1.38± 0.26	1.30± 0.19	1.35± 0.15	1.34± 0.13	1.34± 0.20	N.S.
Constant of anisotropy (K_S)	−2.9	−1.8	−2.4	−2.2	−2.2	
Mean cross sectional area (μm^2)	23.6 ± 3.4	24.4 ± 4.7	23.6 ± 1.4	21.0 ± 2.7	18.2 ± 2.8	$p<0.001$
Myocardial cells						
$L_V \approx Q_A(\alpha=0)$	2637 ±237	2154 ±240	3471 ±289	3585 ±298	2871 ±245	$p<0.001$
Mean cross sectional area (μm^2)	330 ± 31	410 ± 47	246 ± 22	239 ± 20	304 ± 27	$p<0.001$
3D Capillary-fiber-ratio (mm/mm)	1.40± 0.15	1.39± 0.18	1.31± 0.14	1.41± 0.12	1.48± 0.16	N.S.

Means ± standard deviations. Reference volume: myocardial tissue of papillary muscles

$Q_A(\alpha=0)$ and $Q_A(\alpha=\pi/2)$ are the Q_A values in transverse and longitudinal sections, respectively. $B_A(\alpha=0)$ and $B_A(\alpha=\pi/2)$ are the B_A values in transverse and longitudinal sections, respectively. The means of the five groups were tested by one way analysis of variance. A result was considered to be significant if $p<0.05$

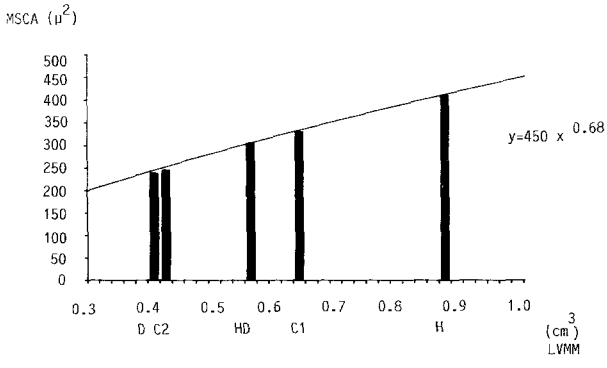

Fig. 6. Correlation between left ventricular muscle cell mass and mean cross sectional area of myocardial cells. The exponential ($0.68 \approx 2/3$) indicates harmonic growth of muscle fibers. C_1: Control group; C_2: Weight-matched control group; H: Hypertensive group; D: Diabetic group; HD: Hypertensive diabetic group

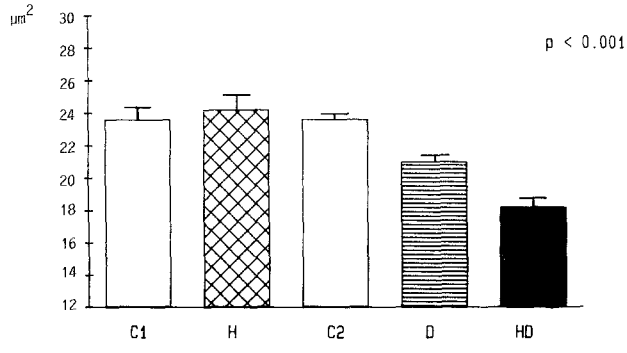

Fig. 7. Mean cross sectional areas of capillaries are decreased in combined diabetes and hypertension. C_1: Control group; C_2: Weight-matched control group; H: Hypertensive group; D: Diabetic group; HD: Hypertensive diabetic group

brils depend mathematically on the volume density of sarcoplasm and the volume ratio mitochondria-to-myofibrils. c) The surface-to-volume ratio of mitochondria is considerably increased in the hypertensive diabetic group as compared to the other groups.

5. Significant differences between the control group and both the weight-matched control group and the diabetic group are detected in length and surface densities of capillaries, and length densities and mean cross sectional areas of myocardial cells (Tables 3, 5). Furthermore, the volume ratio mito-

Table 4. Volume densities (V_V) of interstitial tissue components and myocardial cells (combined light and electron microscopy)

Parameter	Control group $n=12$	Hypertensive group $n=12$	Weight-matched control group $n=7$	Diabetic group $n=10$	Hypert. diabetic group $n=11$	Level of statist. signific.
Myocardial cells (cm^3/cm^3)	0.871 ± 0.021	0.883 ± 0.028	0.853 ± 0.023	0.856 ± 0.015	0.872 ± 0.017	$p<0.05$
Capillary lumen (cm^3/cm^3)	0.087 ± 0.012	0.068 ± 0.020	0.107 ± 0.010	0.106 ± 0.015	0.077 ± 0.015	$p<0.001$
Endothelial cells (cm^3/cm^3)	0.018 ± 0.003	0.019 ± 0.003	0.021 ± 0.002	0.020 ± 0.002	0.021 ± 0.003	N.S.
Interstitial cells (cm^3/cm^3)	0.013 ± 0.005	0.020 ± 0.009	0.011 ± 0.007	0.008 ± 0.002	0.018 ± 0.008	$p<0.005$
Non-cellular interstitial matrix (cm^3/cm^3)	0.011 ± 0.004	0.010 ± 0.005	0.009 ± 0.004	0.010 ± 0.002	0.012 ± 0.003	N.S.

Means $\pm$ standard deviations

The means of the five groups were tested by one way analysis of variance
A result was considered to be significant if $p<0.05$

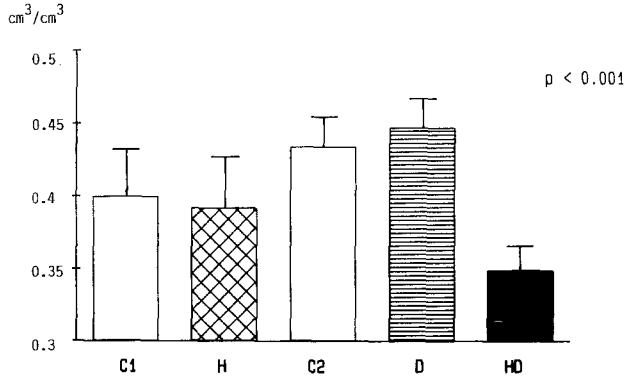

Fig. 8. The volume ratio mitochondria-to-myofibrils is decreased in the hypertensive diabetic group. Note that the ratio tends to be higher in animals with low body weights, i.e. in the weight-matched control group and the diabetic group. However, the reduction in hypertensive diabetic rats occurs despite the low body weights in this group. C_1: Control group; C_2: Weight-matched control group; H: Hypertensive group; D: Diabetic group; HD: Hypertensive diabetic group

Table 5. Significant divergences between the groups Stereological parameters of myocardial cells and interstitial tissue (Scheffe's test)

	C_1–H	C_1–C_2	C_2–D	C_2–HD	D–HD
Capillaries					
L_V	$\downarrow p<0.01$	$\uparrow p<0.001$	N.S.	N.S.	$\downarrow p<0.01$
S_V	$\downarrow p<0.05$	$\uparrow p<0.001$	N.S.	$\downarrow p<0.01$	$\downarrow p<0.01$
V_V	N.S.	N.S.	N.S.	$\downarrow p<0.01$	$\downarrow p<0.01$
Mean cross sect. area	N.S.	N.S.	N.S.	$\downarrow p<0.05$	N.S.
Myocardial cells					
L_V	$\downarrow p<0.001$	$\uparrow p<0.001$	N.S.	$\downarrow p<0.001$	$\downarrow p<0.001$
V_V	N.S.	N.S.	N.S.	N.S.	N.S.
Mean cross sect. area	$\downarrow p<0.001$	$\uparrow p<0.001$	N.S.	$\downarrow p<0.001$	$\downarrow p<0.001$
Interstitial cells					
V_V	N.S.	N.S.	N.S.	N.S.	$\uparrow p<0.05$

$\downarrow$: Significant decrease; $\uparrow$: Significant increase; N.S.: $p \geq 0.05$; C_1: Control group; H: Hypertensive group; C_2: Weight-matched control group; D: Diabetic group; HD: Hypertensive diabetic group

chondria-to-myofibrils tends to be higher in the weight-matched control group ($p<0.1$) and is significantly higher in the diabetic group ($p<0.05$) as compared to the control group (Tables 6, 7). The surface-to-volume ratio of mitochondria is lower in the weight-matched control group ($p<0.05$) when compared with the control group.

Discussion

A weight-matched control group was inferred in the present investigation since the normal growth of the diabetic and hypertensive diabetic rats was markedly suppressed. Differences in length and surface density of capillaries and length density of myocardial cells between the control group and the weight-matched control group and even the diabetic group may be explained by the different mean cross sectional areas of muscle cells. In contrast, the lower surface-to-volume ratio of mitochondria in the control group when compared with the weight-matched control group is obviously not related to the geometry of myocardial cells. A correlation with the heart weight, however, was also detected in a recently published study on thiamine-deficient rats (Mall et al. 1986a). The functional significance of this is not yet known.

The heart weights of the diabetic rats were considerably lower when compared with the control group. However, analysis of heart-to-body weight

Table 6. Stereological parameters of myocardial cell organelles

Parameter	Control group $n=12$	Hypertensive group $n=12$	Weight-matched control group $n=7$	Diabetic group $n=10$	Hypert. diabetic group $n=11$	Level of statist. signific.
V_V myofibrils (cm^3/cm^3)	0.642 ± 0.019	0.636 ± 0.023	0.622 ± 0.021	0.577 ± 0.015	0.645 ± 0.023	$p<0.001$
V_V mitochondria (cm^3/cm^3)	0.257 ± 0.021	0.249 ± 0.022	0.270 ± 0.013	0.258 ± 0.014	0.225 ± 0.012	$p<0.001$
V_V mitochondria/ V_V myofibrils (cm^3/cm^3)	0.400 ± 0.036	0.392 ± 0.039	0.434 ± 0.028	0.447 ± 0.022	0.349 ± 0.021	$p<0.001$ $p<0.001$
S_V ratio mitochondria[a] (m^2/cm^3)	8.31 ± 0.50	7.84 ± 0.32	7.64 ± 0.25	8.06 ± 0.34	8.94 ± 0.52	$p<0.001$
V_V sarcoplasmic matrix (cm^3/cm^3)	0.101 ± 0.006	0.115 ± 0.009	0.108 ± 0.010	0.165 ± 0.021	0.135 ± 0.022	$p<0.001$

Means $\pm$ standard deviations

The means of the five groups were tested by one way analysis of variance. A result was considered to be significant if $p<0.05$

[a] $\{B_A(\alpha=0)/B_A(\alpha=\pi/2)\}<1.2$

Table 7. Significant divergences between the groups Stereological parameters of myocardial cell organelles Scheffe's test

	C_1–H	C_1–C_2	C_2–D	C_2–HD	D–HD
V_V myofibrils	N.S.	N.S.	$\downarrow p<0.01$	N.S.	$\uparrow p<0.001$
V_V mitochondria	N.S.	N.S.	N.S.	$\downarrow p<0.001$	$\downarrow p<0.001$
V_V mitochondria/V_V myofibrils	N.S.	N.S.	N.S.	$\downarrow p<0.001$	$\downarrow p<0.001$
S_V ratio mitochondria	N.S.	$\downarrow p<0.05$	N.S.	$\uparrow p<0.001$	$\uparrow p<0.001$
V_V sarcoplasm	N.S.	N.S.	$\uparrow p<0.001$	$\uparrow p<0.01$	$\downarrow p<0.01$

$\downarrow$: Significant decrease ($p<0.05$); $\uparrow$: Significant increase ($p<0.05$); N.S.: $p \geq 0.05$; C_1: Control group; H: Hypertensive group; C_2: Weight-matched control group; D: Diabetic group; HD: Hypertensive diabetic group

ratios shows that the reduced heart weight in the diabetic groups is caused by the reduced body weight alone and does not reflect a specific cardiac atrophy induced by diabetes mellitus (Table 1). Furthermore, heart-to-body weight ratios demonstrate a similar degree of hypertrophy in hypertensive diabetic and hypertensive nondiabetic rats. It should be emphasized that the differences in mean cross sectional areas of myocardial cells between the five groups correspond to harmonic fiber size increase (Korecky and Rakusan 1978).

The present study was designed to establish possible synergistic effects of diabetes mellitus and hypertension. Whereas qualitative morphological investigation failed to substantiate such effects the quantitative stereological parameters show three microstructural reactions which are detected in the hypertensive diabetic group alone: 1) a decreased volume ratio of mitochondria-to-myofibrils, 2) an increased surface-to-volume ratio of mitochondria, and 3) decreased mean cross sectional areas of capillaries.

In hypertrophy induced by chronic pressure overload, a decreased volume ratio of mitochondria-to-myofibrils was described in the early sixties by Wollenberger and Schulze (1962) and a long series of investigations has hitherto confirmed this reaction pattern of myocardial cell organelles (Poche et al. 1968; Meerson et al. 1964; Anversa et al. 1971, 1980; Lund and Tomanek 1978; Wiener et al. 1979; Wendt-Gallitelli et al. 1979; Breisch et al. 1980; Tomanek and Hovanec 1981; Dämmrich and Pfeifer 1983; Rakusan and Tomanek 1986). It should be emphasized, however, that it was not observed in hypertrophy induced by physical exercise (Bozner and Meesen 1969; Frenzel 1985b; Mattfeldt et al. 1986) or by chronic sideropaenic anaemia (Datta and Silver 1975). It is now generally accepted that it depends on both severity and duration of pressure overload (Breisch et al. 1980; Mall et al. 1986b). The relative decrease in mitochondrial mass may reflect a diminished working capacity of the hypertrophic heart (Wollenberger and Schulze 1962; Meerson et al. 1964; Poche et al. 1968; Wendt-Gallitelli et al. 1979) which may contribute to cardiac insufficiency in long-standing hypertension.

In contrast, investigations on hearts in streptozotocin-induced diabetes mellitus did not reveal those effects on cardiac ultrastructure. Fischer et al. (1981) found normal ultrastructure even in long-standing streptozotocin-induced diabetes

mellitus. Never-the-less, several groups have observed loss of myofibrils and sarcoplasmic oedema (Frenzel 1985a; Jackson et al. 1985; Hsiao et al. 1987). The increased volume density of sarcoplasm and the decreased volume density of myofibrils which we found in the diabetic group agrees with the latter findings. However, the reduced volume density of myofibrils in our early stage diabetic animals may to explained by a relative reduction in myofibrillar mass following sarcoplasmic oedema rather than with regressive changes of myofibrils. Otherwise, the hypertensive-diabetic rats would probably not increase the relative mass of myofibrils. The reduced volume ratio of mitochondria to myofibrils in the hypertensive diabetic group which is not present in the hypertensive group provides evidence that the typical quantitative reactions of the myocardial cell organelles in chronic pressure overload occur earlier in diabetic than in non-diabetic rats. This is also supported by the increased surface-to-volume ratio of cardiac mitochondria in the hypertensive diabetic group which is probably related to the presence of smaller mitochondria (Mall et al. 1980). A reduced size of mitochondria is a common phenomenon in cardiac hypertrophy induced by chronic pressure overload (Poche et al. 1968; Anversa et al. 1971). Thus our study indicates for the first time that diabetes mellitus magnifies the myocardial cell alterations in chronic hypertension.

Diabetes mellitus is associated with slightly reduced serum concentrations of thyroid hormones (Fein et al. 1980). Severe hypothyroidism induced by thyroidectomy decreased the volume ratio of mitochondria to myofibrils but does not change the mitochondrial size (Smith and Page 1976). Thus it is unlikely that the synergistic effects in hypertensive diabetic animals are caused by the slight hypothyroidism.

The molecular basis of the ultrastructural processes is not known. A decrease in myofibrillar ATPase activity and increase in V_3 isomyosins are induced by hypertension (cf. Jacob 1983) as well as by diabetes mellitus (Dillmann 1980; Fein et al. 1980; Pierce and Dhalla 1981; Rubinstein et al. 1984; Bimji et al. 1986), but there are no reports on a combination of hypertension and diabetes. Cardiac contractility is decreased in diabetic (Penpargkul et al. 1980; Jackson et al. 1985; Frenzel et al. 1985a), hypertensive (cf. Jacob 1983) and hypertensive diabetic rats (Fein et al. 1984).

Recent investigations have shown pronounced replacement fibrosis and interstitial fibrosis in hearts of hypertensive diabetic humans even in the absence of significant alterations of the extramural coronary vessels and similar changes were described in hypertensive diabetic rats after 8 weeks (Factor et al. 1980, 1981). The occurrence of a hypertensive diabetic cardiomyopathy has been postulated since the fibrosis was more pronounced in hypertensive diabetics as compared to hypertensives. In the present study, fibrosis could not be established 8 weeks after clipping the left renal artery and 4 weeks after injection of streptozotocin. However, the volume density of interstitial cells was slightly increased in the hypertensive and the hypertensive diabetic group. This may reflect an early stage of activation of interstitial cells which leads to interstitial fibrosis at later stages (Thiedemann et al. 1983). The absence of fibrotic changes in the heart was paralleled by an unchanged thickness of glomerular basement membranes in the unclipped kidney (unpublished observations).

However, in hypertensive diabetic animals we found a reduced cross sectional area of capillaries. This may be related to impaired cardiac microcirculation but functional data on the myocardial blood flow in hypertensive diabetic animals have not yet been published. We suggest that two mechanisms are to be taken into consideration. Firstly, an increased resistance of small arteries and arterioles may lead to lower capillary pressures and secondly molecular changes in the interstitial tissue components (including basement membranes) may reduce the distensibility of capillaries. Both hypotheses are supported by observations of Factor et al. (1981) who found in hypertensive diabetic rats a pronounced replacement fibrosis which may be due to vascular spasms and a pronounced non-replacement fibrosis.

Flückiger et al. (1984) presented a careful investigation on spontaneously hypertensive rats after long-standing diabetes and could not detect significant myocardial fibrosis. In the rat model, pronounced interstitial fibrosis may therefore be restricted to diabetic rats with renovascular hypertension.

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