Limited Adaptation in Chronically Hypertrophied Hearts from Aortic Constricted Rats: Increased Inhomogeneity in Cross-Sectional Area of Cardiomyocytes and Intercapillary Distance

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Suzuki, Y., Harada, K., Kawamura, K., Masuda, H. and Takada, G. Limited Adaptation in Chronically Hypertrophied Hearts from Aortic Constricted Rats: Increased Inhomogeneity in Cross-Sectional Area of Cardiomyocytes and Intercapillary Distance. Tohoku J. Exp. Med., 1993, 170 (3), 181-195 — To study the sequential changes of myocardial structure in pressure-overloaded heart, rats were subjected to banding of ascending aorta at 8 weeks of age, and electron-microscopical morphometry was performed at weeks 1, 2, 4, 8, 16, and 24 postoperatively. Not only was the cross-sectional area of cardiomyocyte greater in banded rats but the degree of inhomogeneity increased in correspondence with development of hypertrophy. Intercapillary distance was significantly longer in banded rats than in controls. In banded rats, the distance around larger-than-normal cells (with a cross-sectional area exceeding 600 μm²) was significantly longer than that around normal-sized cells (with a cross-sectional area under 600 μm²) at 4, 16, and 24 weeks following surgery. Morphologically, intracellular capillaries were shown to develop; however, single or several necrotic cells were observed in 8- to 16-week banded rats and were attributed to acute ischemia. In 24-week banded rats, advanced interstitial fibrosis as well as collapsed intracellular capillaries were observed. ——— cardiac hypertrophy; electron microscopy; morphometry; aortic constriction

Pressure overload of the heart generally results in concentric hypertrophy (Linzbach 1960; Grossman et al. 1975; Lin Lin et al. 1977) characterized by increased cross-sectional areas of cardiomyocytes (Grossman et al. 1975; Marcus et al. 1982; Anversa et al. 1986). This is considered an adaptive response in which increased sarcomeres are laid down in parallel (Grossman et al. 1975; Coffelt et al. 1979). In left ventricular hypertrophy due to aortic constriction, changes in myocardial tissue composition have been reported to be similar to that associated with normal growth (Laase et al. 1988). Structural changes were suggested to play a role in maintaining left ventricular function (Imamura et al. 1990).
Nevertheless, if pressure overload is severe and longstanding, hypertrophy becomes pathological in nature (Coffelt et al. 1979; Weber et al. 1987). At this stage coronary blood flow reserve is diminished (Marcus et al. 1982; Bache et al. 1984) and diastolic compliance as well as systolic function reduced (Peterson et al. 1978; Krayenbuehl et al. 1983). Obviously, in cardiac hypertrophy there is a limit to overload as a process of adaptation (Swynghedauw 1989). The mechanism by which left ventricular hypertrophy may reach the limit is unclear and probably multifactorial, however, myocardial ischemia has been suggested to be an important component (Weber et al. 1987). Morphometric studies in animal models have demonstrated an increased inhomogeneity of cardiomyocyte size as well as a prolonged intercapillary distance in this pathological condition (Rakusan et al. 1986). Also, it has been reported that tissue oxygenation was influenced by capillary spacing (Turek and Rakusan 1981) and myocyte loss occurred in hypertrophied heart (Campbell et al. 1991). However, the relation between the inhomogeneity of myocyte size and capillary spacing has not been clear and the cause of myocyte loss has not been demonstrated. Hitherto, in most studies cardiomyocyte size was measured using a light microscope (Lund and Tomanek 1978; Dämmrich and Pfeifer 1983; Kuribayashi et al. 1986; Laase et al. 1989) and intercapillary distance estimated by capillary density (Rakusan 1971; Gerdes et al. 1979; Dämmrich and Pfeifer 1983; Rakusan et al. 1986). In this study, morphometry in animal models of long-term pressure overload was done on very large low power electron micrographic pictures to understand the correlation between myocyte size and intercapillary distance in detail. Also, morphologic study in large number of cells was done for qualitative analysis of myocyte loss.

**MATERIALS AND METHODS**

Fifty-eight Sprague-Dawley rats (8 weeks old, 295±10 g) were used. Twenty-eight rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally and were intubated through a tracheostomy. The left thorax was opened at the third intercostal space to expose the ascending aorta under artificial ventilation with room air. The ascending aorta was dissected free and a surgical silk thread (4-0) was drawn under the ascending aorta. A rigid tube (4F NIH Catheter, 1.4 mm in o.d.) was placed alongside the ascending aorta. The rigid tube and the ascending aorta were tied tightly together with the thread. Then, the rigid tube was removed rapidly, leaving the ascending aorta constricted to a diameter of 1.4 mm. Thereafter, animals were kept for 1, 2, 4, 8, 16 and 24 weeks. Five age-matched control animals were used in each group. At sacrifice, a cannula was introduced into the abdominal aorta under pentobarbital anesthesia and the heart was washed out with 75 ml of oxygen-saturated heparinized lactate Ringer’s solution. Then 50 ml of 3% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) was perfused under 100 mmHg at room temperature.

After removing both atria and the free part of the right ventricular myocardium, the wet weight of the left ventricle was determined including the ventricular septum. A transverse slice was taken from the middle layer of the myocardium at the level of the upper one-third of the left ventricle. For electron microscopic study, both exactly transverse and longitudinal specimens were prepared from the slice. These were post-fixed with 1% osmic acid, dehydrated using alcohol and embedded in Epon 812 resin.
Super wide mesh (grid size: 0.5 x 0.67 mm) was used for large low power electron micrographic pictures. The ultrathin sections were observed with a LEM 2000 transmission electron microscope. A wide grid field was continuously exposed using the panorama-photo-system (LM-PVP). Then a panorama photograph was composed from 24 TEM photographs (4 x 6 sheets, 70 cm x 100 cm, ×1600 magnification). On photographs of transverse section, the cross-sectional area of cardiomyocyte and the center-to-center distance of all the neighboring capillaries around one myocyte were measured for 150 to 250 cells in each sample with the aid of a computer-interfaced digitizer pad (Nikon COSM-OZONE 1S).

When no Z-line was observed in an electron micrograph, as in Fig. 1A, the muscle fibers were judged to be showing cross sections. On the other hand, when rows of Z-lines were observed as in Fig. 1B, we considered that the muscle fibers were cut in oblique direction. In the latter case, the average distance between a pair of neighboring Z-lines was measured (a in Fig. 1B) for randomly chosen six cells. Next, the average sarcomere length was measured in TEM photograph of longitudinal section (Fig. 1B) on a series of 10 sarcomeres (b in Fig. 1B). From the ratio b/a, the angle of muscle fiber inclination was calculated, which allowed us to correct the cross-sectional area, assuming a cylindrical configuration (Korecky and Rakusan 1978).

Fig. 1. If muscle fiber was cut cross-sectionally, Z-lines were not observed in TEM photograph of transverse section (A). In contrast, if muscle fiber was cut oblique sectionally, rows of Z-lines were observed (B). a, distance between neighboring Z-line. b, sarcomere length. * an angle of inclination
All the data were subjected to the analysis of variance (ANOVA), which was followed by Student's t-test to evaluate the level of significance between age matched groups. The frequency distribution was analyzed by $X^2$ test. $p$-values less than 0.05 were considered significant.

The protocols for animal experimentation described in this paper were previously approved by the Animal Research Committee, Akita University School of Medicine; all subsequent animal experiments adhered to the "Guidelines for Animal Experimentation" of the University.

RESULTS

Neither congestion of the liver nor a pleural effusion was observed at autopsy of the animals.

Body weight at sacrifice did not differ significantly between the banded rats and controls (Fig. 2). The cross-sectional area of the ascending aorta was constricted to 19.7% ± 0.5% of the aortic root; no significant difference was observed in each group. The left ventricular weight of experimental rats increased significantly as early as 1 week after surgery compared with control rats. The left ventricular weights in banded rats attained a plateau at 16-24 weeks after surgery (Fig. 3).

The mean cross-sectional area of cardiomyocytes was significantly greater in banded rats than in control (Fig. 4). In Fig. 5, the distributions of the cross-sectional myocyte areas are compared between the control and banded rats. In control, the distribution curves were narrow and positioned at low values (Fig. 5a). In all the age groups of controls, the cross-sectional area was under 600 $\mu$m$^2$ in more than 99% of myocytes. Thus, a cross-sectional area under 600 $\mu$m$^2$ was shown to serve as a standard for normal adult rats. In contrast, in banded rats, the distribution curves were significantly shifted to relatively higher levels in correspondence with the progress of cardiac hypertrophy ($p < 0.01$) and there was

![Fig. 2](image)

Fig. 2. Body weight is not significantly different between aortic constricted rats (●) and controls (○). (ANOVA) Values are means, bars = SEM.
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...cells co-exist with larger-than-normal cells in myocardium of banded rats. The intercapillary distance of each group is summarized in Table 1. Intercapillary distance increased significantly in banded rats at all follow-up periods except at 1 week after surgery. Then myocytes of each banded group except for 1 week and 2 weeks after surgery were divided into two subgroups, in which the cross-sectional area was under or over $600 \, \mu m^2$. The cross-sectional area of almost all myocytes in 1- and 2-week banded rats were under $600 \, \mu m^2$. Next, the intercapillary distance around the larger-than-normal cells and the normal-sized cells was measured separately. The intercapillary distance around the larger-
Fig. 5a, 5b. Frequency distribution of cross sectional area of myocyte. Average values for the controls (5a) and aortic-constricted rats (5b). c, \( p < 0.01 \), significance of the \( \chi^2 \) test done for the six groups of normal heart. d, \( p < 0.01 \), significance of the \( \chi^2 \) test done for the six groups of aortic constricted heart

**Table 1. Intercapillary distance in each group**

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<td>AoC</td>
<td>Mean</td>
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<td>17.1**</td>
<td>17.9**</td>
<td>18.3**</td>
<td>19.1****</td>
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<td>4.5</td>
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<td>4.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Cont</td>
<td>Mean</td>
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<td>15.0</td>
<td>15.9</td>
<td>15.9</td>
<td>15.4</td>
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<tr>
<td></td>
<td>S.D.</td>
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<td>3.4</td>
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AoC, Aortic constricted group; Cont, Age matched control group; \(^*p < 0.006\) significant effect of aortic constriction (ANOVA); \(^*p < 0.05; **p < 0.01\), for comparison of means between aortic constricted group and control group.
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Table 2. Intercapillary distance in aortic constricted rats

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<thead>
<tr>
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<tr>
<td>AoC_L</td>
<td>19.1*</td>
<td>18.1</td>
<td>19.3*</td>
<td>19.5* *b</td>
<td></td>
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<tr>
<td>(\mu m)</td>
<td>5.3</td>
<td>5.3</td>
<td>6.0</td>
<td>5.9</td>
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<td></td>
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<tr>
<td>AoC_N</td>
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<td>17.2</td>
<td>17.5</td>
<td>16.8</td>
<td></td>
<td></td>
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<tr>
<td>(\mu m)</td>
<td>4.9</td>
<td>4.2</td>
<td>5.0</td>
<td>5.1</td>
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AoC_L, Intercapillary distance around larger-than-normal cells (55±8 cells) in aortic constricted rat; AoC_N, Intercapillary distance around normal-sized cells (53±5 cells) in aortic constricted rat; \*p < 0.05 significant difference which is attributed to myocyte size (ANOVA), \*p < 0.05 for comparison of means between AoC_L and AoC_N.

than-normal cells was significantly longer than that around the normal-sized cells at 4, 16, and 24 weeks after surgery (Table 2).

Occasionally, single cell necrosis was observed in 8-week banded rats (Fig. 6). In 16-week banded rats, three necrotic myocytes including myofibrillar degeneration, infiltration of macrophages, and myocytolysis were observed (Fig. 7). In 24-week banded rats, interstitial fibrosis progressed and the formation of deep transverse groove containing capillary in their lumen was seen in hypertrophied myocyte (Fig. 8). In a few instances, hypertrophied myocyte contained one or two narrow tunnels of sarcolemmal membrane within the sarcoplasm. The intracellular tunnels usually contained one capillary in one lumen. These tunnels were relatively rare (1.5±0.3\%) in 8-week banded rats but increased (3.2±0.4\%) in 16-week banded rats (Fig. 9). In 24-week banded rats, some intracellular capillaries appeared to have no function because of collapsed lumen and thin endothelial cells (Fig. 10).

DISCUSSION

In the adult rat, increased pressure load induces concentric ventricular hypertrophy of heart in which wall thickness increases (Kuribayashi et al. 1986). This adaptational process should offset higher peak systolic wall stress (Grossman et al. 1975) and cardiac function was reported to be preserved in early and late phases of exposure to pressure overload (Imamura 1990). However, it is pointed out that there are biological limitations to this process in that the myocyte cannot continually hypertrophy (Swynghedauw 1989). In this study, the increase in left ventricular weight reached a plateau between 16 to 24 weeks after surgery. These results suggested that hypertrophic response reached a critical point at 16–24 weeks after surgery.

This study has demonstrated that in hypertrophic heart, the cross-sectional area of cardiomyocyte not only increases but also becomes inhomogeneous. Although the inhomogeneity of myocyte size was shown to increase with age even in normal heart, the degree of inhomogeneity was far higher in aortic constricted
Fig. 6. Single necrotic myocyte in 8-week postoperative rat. Myofibril disappeared and cellular infiltration (↑) was observed. ×2,500. Scale bar = 10 μm
Fig. 7. Three necrotic myocyte in 16-week postoperative rat. Degenerated myofibril (*), infiltration of macrophages (►), and myocytolysis (**) were observed. ×2,000. Scale bar = 10 μm
Fig. 8. Myocyte in 24-week postoperative rat. Progressed interstitial fibrosis was observed. The formation of deep transverse groove (↓) was seen in hypertrophied myocyte. This groove contained capillary in their lumen. ×2,000. Scale bar = 10 μm.
Fig. 9. Intracellular capillary runs through intracellular tunnel (↓) in hypertrophied myocyte in 16-week postoperative rat. ×4,000. Scale bar=5 μm
Fig. 10. Degenerated intracellular capillary (↑) in 24-week postoperative rat. Collapsed lumen and thin endothelial cell were observed. ×9,000. Scale bar = 2 μm
heart where it continued to increase with the development of hypertrophy. The mechanisms responsible for the great inhomogeneity of myocyte size in the myocardium of hypertrophic heart are at present unknown. It seems however that the growth potential differs in individual myocytes, which becomes more obvious in hypertrophy by pressure overload. Campbell et al. (1991) reported that heterogeneity in myocyte size increased after abdominal aortic constriction and they considered that smaller myocyte had the highest growth potential. In the same report the distribution curves for myocyte size were described not to change in cardiac hypertrophy due to experimental hyperthyroidism. The myocardium is likely to respond differently depending on the nature of the hemodynamic stress.

It is reported that capillary proliferation does not occur in pressure overloaded hypertrophy (Anversa et al. 1978, 1986). In contrast, the average size of capillaries has been reported to be consistently larger (Capasso et al. 1990). An increased cross-sectional area of myocyte also means an increase in the diffusion distance between capillaries and the center of hypertrophic cells. This impairs the diffusion of substances, notably oxygen essential for the production of energy. Weber et al. (1987) suggested that an abnormally long diffusion path underlies the pathophysiology of cardiac hypertrophy. The transverse groove seen in Fig. 7 in this study may be formed as a result of ischemia in the center of hypertrophic cell. On the other hand, Kawamura et al. (1976) found “intracellular capillaries” in spontaneously hypertensive rats, which become more numerous with the development of hypertrophy. They considered that the formation of these myocardial-cell-penetrating capillaries represented an adaptative phenomenon, relieving the centers of hypertrophied cells from the impeded oxygen diffusion. In this study, intracellular capillaries were also observed in the advanced stage of cardiac hypertrophy. Thus, capillary proliferation, although not very often, occurs in pressure overloaded hypertrophy. However, not only interstitial fibrosis progressed, but also some of the intracellular capillaries were destroyed after the left ventricular weights reached a plateau. The reason why these capillaries collapsed is unknown. At least capillaries do not appear to be susceptible to proliferation in pressure overloaded hypertrophy.

Rakusan et al. (1986) reported that not only did average intercapillary distances increase but also that the degree of inhomogeneity in their spacing was significantly higher in hypertrophic hearts. Also, tissue oxygenation is reported to be influenced not only by the mean values of the intercapillary distance but also by inhomogeneity of the capillary spacing. The results of this study demonstrate that the intercapillary distance around larger-than-normal cells is longer than normal-sized cells in banded rats. This fact might mean the inhomogeneity in capillary spacing. Also, oxygenation of larger-than-normal cell could be impaired more than normal-sized cell.

Myocyte loss and collagen accumulation have recently been shown to play an
essential role in the development of dysfunction in long-term hypertension (Capasso et al. 1990) and aging (Anversa et al. 1990). Campbell et al. (1991) reported that some level of myocyte loss occurred also in rats with aortic constriction. As a cause of myocyte loss, local alteration in myocardial perfusion (Rakusan et al. 1980), alterations in cellular membrane properties with calcium overloading (Buja et al. 1990), or catecholamine-induced myocyte necrosis (Benjamin et al. 1989) have been considered in past studies. In this study, single-or several-cell necrosis including myofibrillar degeneration, infiltration of macrophages, and myocytolysis proved to occur in progressing stages of cardiac hypertrophy. These changes correspond to acute ischemic damage. Considering the inhomogeneity of myocyte size and capillary spacing in hypertrophic heart, we speculate that ischemia is one of the factors responsible for the loss of individual myocytes.

In conclusion, inhomogeneity of myocyte size and capillary spacing that develops in the advanced stage of cardiac hypertrophy is important in that it causes myocyte loss. Although some capillaries proliferate so as to form intracellular tunnels, they do not appear sufficient to maintain the oxygenation of myocytes. Myocytes in which a balance was not sustained between the supply and demand of O₂ may be destroyed just as noted in acute ischemia.

References


