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The Effects of Beta Adrenoceptor Blockade With Atenolol on Myocardial Cellular and Subcellular Hypertrophy in Spontaneously Hypertensive Rats

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ABSTRACT In this study we investigated the effects of chronic β adrenoreceptor blockade with atenolol on cellular and subcellular hypertrophy in spontaneously hypertensive rats (SHR). Atenolol was injected subcutaneously (20 mg/kg) twice daily commencing in four-week-old rats. The treated animals (SHR-A) were compared to their nontreated controls and normotensive, Wistar-Kyoto (WKY) controls at the age of 16 weeks. A group of atenolol-treated WKY was also studied. Chronic drug treatment was effective in attenuating the rise in systolic blood pressure characteristic of SHR, but did not normalize the values to those of WKY. Cardiac hypertrophy, characteristic of SHR, was modified by drug treatment as evidenced by left ventricular weights as well as myocardial cell size. The cells from the subendocardium underwent selective hypertrophy in SHR which was attentuated by about 50% after atenolol treatment. Stereological analysis of electron micrographs showed that while relative mitochondrial volume was not affected by treatment, relative myofibrillar volume (%) decreased in both subepicardium (SHR = 63.28 ± 1.25; SHR-A = 56.72 ± 1.37) and subendocardium (SHR = 66.53 ± 1.27; SHR-A = 58.30 ± 1.51). This change raised the mitochondrial/myofibrillar volume ratio, which is characteristically low in SHR compared to WKY. Sarcoplasm, which included all cell constituents except mitochondria, increased with atenolol treatment, but water concentration remained unchanged. The data suggest that attenuation of hypertrophy in SHR after β blockade is associated with selective effects on the myocardial cell involving primarily the myofibrillar cell compartment.

Left ventricular hypertrophy in the spontaneously hypertensive rat (SHR), a popular model of essential hypertension, is similar to that found in other models of pressure overload. Quantitative changes in cellular constitutents are characterized by a decrease in the volume ratio: mitochondria/myofibrils (Lund and Tomanek, 1978; Tomanek et al., 1979; Tomanek, 1979a,b; Tomanek and Hovanec, 1981). A decrease in this ratio due to a relative increase in myofibrillar volume and/or a relative decrease in mitochodrial volume has also been found in other forms of pressure overload, i.e., aortic constriction (Page et al., 1972; Goldstein et al., 1974; Anversa et al., 1976; Lund and Tomanek, 1978) and renal artery constriction (Wiener et al., 1979; Wendt-Gallitelli and Jacob, 1977). Such a decrement in the ratio of energy-producing to energy-consuming organelles could presumably limit myocardial metabolism under conditions requiring high O_2 utilization.

While the increased afterload appears to be the major stimulus for cardiac hypertrophy due to pressure overload, the direct stimulus at the cellular level is not evident at this time. The role of factors other than the pres-

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sure overload itself, at least in SHR, is suggested by studies which have shown that cardiac hypertrophy can 1) develop in the absence of hypertension (e.g., when hydralazine is used to control blood pressure; Sen and Bumpus, 1979), and 2) be prevented with α-methyldopa even at dosages which do not prevent the rise in blood pressure characteristic of SHR (Tomanek et al., 1979). Recent work in our laboratory (Tomanek et al., 1982) has shown that catecholamines are not the determining factors in the magnitude of cardiac hypertrophy in SHR, but rather appear to regulate myofibril volume. These findings, then, indirectly implicate β_1 adrenergic cardiac receptors in disproportional organelle growth characteristic of cardiac hypertrophy in SHR.

The study described here was initiated to test the hypothesis that blockade of these receptors with the β_1 antagonist atenolol would normalize the relative volumes of cellular constituents. This approach permitted us to explore the role of β_1 receptors in regulating the quality and/or quantity of cardiac hypertrophy in SHR.

MATERIALS AND METHODS

Male SHR and their genetic, normotensive counterparts, Wistar-Kyoto (WKY), were used in this study. Atenolol was injected (20 mg/kg, subcutaneously) twice daily beginning at 4 weeks of age and continuing until the animals were studied at 16 weeks of age. Control animals received equivalent doses of saline. The animals were maintained on a 12-hour light/dark cycle and had free access to food and water. Blood pressure was monitored twice weekly by the tail-cuff method (Tomanek et al., 1979).

At the end of the experimental period the animals were anesthetized with Penthrane, mechanically ventilated, and a midline thoracotomy was performed. After the great vessels were dissected free of connective tissues, heparin sulfate (5,000 units) was injected via the inferior vena cava and allowed to circulate for 1 minute. A cannula, consisting of a 19-gauge butterfly needle, was inserted into the ascending aorta, and the tip positioned just above the coronary ostia; the cannula was secured in place with two silk ligatures. The heart was arrested in diastole by injecting 1 ml of procaine into the left ventricular lumen. The right atrium was cut open to allow perfusate outflow and the heart perfused with 20 cc of Locke's solution containing 1 cc of procaine, heated to 37°C. The wash was followed by approximately 100 ml of a modified Karnovsky fixative solution (Tomanek and Karlsson, 1973).

The heart was excised, blotted dry, and whole heart and left ventricular weight (including the septum) was recorded. The left ventricular free wall was then placed in fresh fixative for an additional 2–4 hours. Under a dissecting microscope the subepi- and subendocardium of the left ventricular free wall were separated and groups of fibers were teased out using microforceps. Samples were removed from each layer and placed in a 0.1 M sodium cacodylate buffer with 3% sucrose for 24–36 hours. The tissue was postfixed in osmium tetroxide (1%) for 2 hours. Samples were rinsed in neutral buffer, dehydrated through a series of ethanols and propylene oxide, and embedded in Epon 812 in flat molds.

Light Microscopy and Morphometrics

One-micron sections were cut on an LKB Huxley microtome, transferred to glass slides, and stained with Richardson's solution. The slides were projected using a Leitz microprojector at a final magnification of ×400. Approximately 20 fibers from each block were traced. To assure that the cell area was measured at approximately mid-level, only those fibers sectioned at the level of the nucleus were traced. Cell cross-sectional areas were subsequently determined with a Talos Image Analyzer which was interfaced to a Monroe 1860 desk calculator. The mean area of each specimen was recorded.

Electron Microscopy and Stereology

Thin cross sections were cut on an LKB Huxley ultramicrotome, mounted on copper grids, and stained at room temperature with uranyl acetate and lead citrate solutions. All specimens were viewed with a Siemen's Elmskop 101 electron microscope at 60 kV. Ten cross-sectional cell profiles from each specimen, representing ten fibers, were photographed. The micrographs were enlarged to a final magnification of ×18,000.

Fractional cell volumes were calculated based on the technique established by Page and McCallister (1973) and routinely used in our laboratory (Tomanek et al., 1979; Tomanek, 1979a). A transparent overlay grid with intercepts every 6 ml was superimposed over each micrographs. Three classifications of

TABLE 1. Body weight and absolute left ventricular weight[1]

Group (N)	Body weight (gm)	Left ventricular wt (mg)
WKY (10)	270 ± 13	566 ± 35
WKY-A (5)	261 ± 18	562 ± 49
SHR (11)	306 ± 10	835 ± 28
SHR-A (13)	294 ± 11	725 ± 30
WKY vs. SHR	NS	< 0.001
WKY vs. WKY-A	NS	NS
SHR vs. SHR-A	NS	< 0.01

[1]All values are mean ± SEM. WKY, Wistar-Kyoto; SHR, spontaneously hypertensive; A, atenolol-treated.

subcellular structures were used: 1) mitochondria, 2) myofibrils, and 3) all other organelles, matrix, and inclusions (sarcoplasm).

Statistical treatment was based on analysis of variance. For those variables with significant F values, the Bonferoni t-test was used to determine significant differences between group means. A probability level of < 0.05 was considered statistically significant.

RESULTS

To verify the effectiveness of atenolol, heart rates were recorded in four SHR and four WKY rats before and after atenolol injection. A resting bradycardia was evident by 18% and 11% declines in heart rate in WKY and SHR, respectively, within 15 minutes of the injection. This bradycardia persisted throughout the 6-hour recording period.

Left Ventricular Mass and Water Content

Table 1 includes body weight and left ventricular weight. At the time of sacrifice, SHR were signficantly heavier than control WKY animals; chronic atenolol treatment did not alter body weight in SHR or WKY. Left ventricular (LV) weight was greater (by 47%) in SHR than WKY, and was markedly modified in the SHR treated with atenolol (SHR-A). LV water percent ($\bar{X} \pm$ SEM) was similar in all groups studied: WKY = 80.4 ± 0.8; WKY-A = 78.3 ± 1.9; SHR = 80.6 ± 0.6; SHR-A = 79.6 ± 0.1.

Blood Pressure and Left Ventricular Weight/ Body Weight Ratio

Values for blood pressure and left ventricular weight/body weight (LVW/BW) are shown in Figure 1. All SHR used in this study had systolic blood pressures greater than 154 mm Hg as measured by the tail-cuff method; the group mean is significantly higher in control SHR when compared to control WKY. Atenolol treatment modified the increase in blood pressure in treated SHR but the mean value remained above normotensive levels.

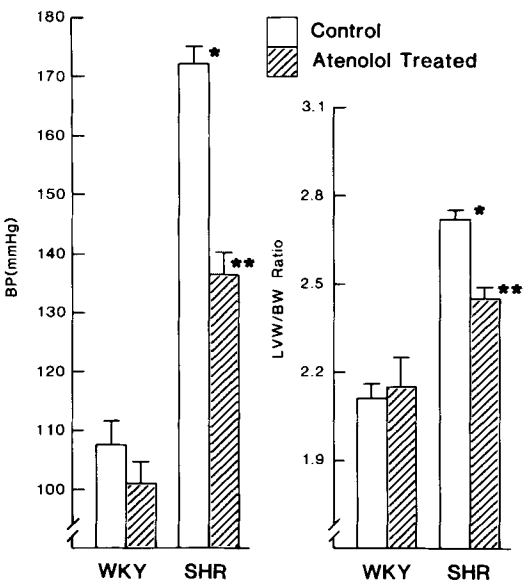

Fig. 1. Systolic blood pressure (BP) and left ventricular weight (mg)/body weight (gm) ratio (LVW/BW) of atenolol-treated and nontreated Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. The number of animals is indicated in Table 1. All values are $\bar{X} \pm$ SEM. Statistically significant differences ($P \leq 0.05$) are indicated: *, SHR vs. WKY; **, atenolol-treated vs. strain control.

Control values for LVW/BW were 29% higher in SHR than in WKY. Drug treatment signficantly attenuated the increase in left ventricular mass in SHR; LVW/BW ratios of SHR treated with atenolol were about 10% lower than their nontreated controls. Left ventricular hypertrophy, though modified in drug-treated animals, remained above that for control WKY animals.

Fiber Area

Although mean fiber areas (Fig. 2) in both regions of the heart were larger in SHR than in WKY, the difference is significant only in the subendocardium. Analysis of the fiber area data shows that the subendocardial fi-

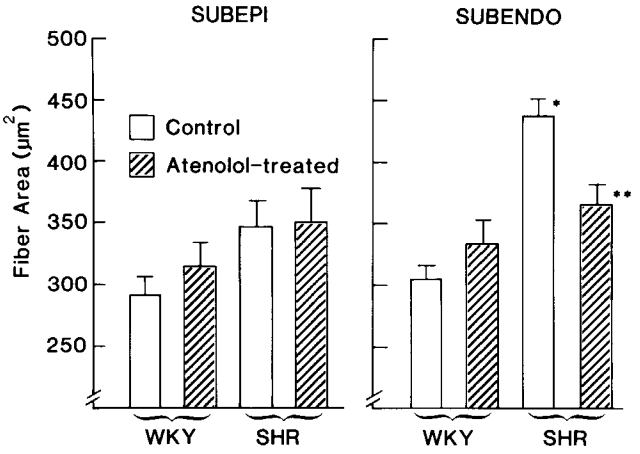

Fig. 2. Cross-sectional areas of fibers obtained from the subepicardium (SUBEPI) and subendocardium (SUBENDO). All values are $\bar{X} \pm$ SEM. The number of animals is provided in Table 2. *, Statistically significant differences between SHR and WKY.

TABLE 2. Relative cell volumes (% of total cell volume) of the three major cellular compartments[1]

Group (N)	Myofibrils	Mitochondria	Sarcoplasm
Subepicardium			
WKY (8)	63.06 ± 1.08	30.69 ± 0.68	6.70 ± 1.11
WKY-A (3)	58.33 ± 1.77	29.83 ± 1.12	12.73 ± 1.69
SHR (6)	63.28 ± 1.25	28.53 ± 0.79	8.17 ± 1.19
SHR-A (5)	56.72 ± 1.37	29.16 ± 0.87	14.04 ± 1.31
WKY vs. SHR	NS	NS	NS
SHR vs. SHR-A	< 0.005	NS	< 0.005
WKY-A vs. WKY	NS	NS	< 0.01
Subendocardium			
WKY (9)	61.84 ± 1.12	32.70 ± 0.94	5.67 ± 0.97
WKY-A (3)	58.30 ± 1.94	29.13 ± 1.63	12.57 ± 1.58
SHR (7)	66.53 ± 1.27	29.26 ± 1.07	4.21 ± 1.03
SHR-A (5)	58.30 ± 1.51	28.26 ± 1.26	14.54 ± 1.22
WKY vs. SHR	< 0.02	NS	NS
WKY vs. WKY-A	NS	NS	< 0.01
SHR vs. SHR-A	< 0.001	NS	< 0.001

[1]All values are percentage of total cell volume expressed as $\bar{X} \pm$ SEM.

bers underwent preferential hypertrophy, i.e., a 43% increase in cross-sectional area. Chronic treatment with atenolol modified fiber growth in the subendocardial region in SHR so that the magnitude of the hypertrophy in this region was approximately 50% of that of the SHR controls. Fiber area in WKY was not significantly affected by atenolol treatment.

Stereology

The subcellular organelle volumes determined by point-counting analysis are provided in Table 2. In subepicardial specimens the relative volumes of mitochondria, myofibrils, and sarcoplasm are not signficantly different in control SHR and WKY. However, atenolol treatment signficantly reduced relative myofibrillar volume in SHR below the control value and conversely increased relative sarcoplasmic volume. Mitochondrial volume, however, was not altered by drug treatment. In WKY treated with the beta blocker, the only change was an increase in sarcoplasm similar to that which occurred in treated SHR.

In the subendocardium, where significant hypertrophy occurred, relative myofibrillar

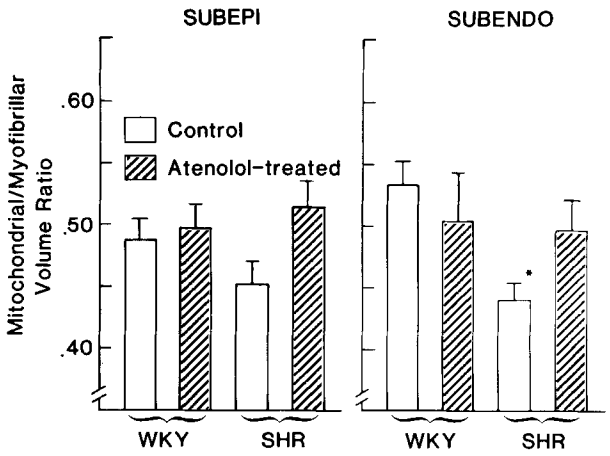

Fig. 3. Mitochondrial/myofibrilar volume ratio of fibers obtained from the subepicardium and subendocardium. All values are $\bar{X} \pm$ SEM. The number of animals is provided in Table 2. *, Statistically signficant difference between SHR and WKY.

volume is significantly greater in control SHR than in WKY. The growth of myofibrillar volume is significantly lower in treated SHR when compared with their SHR controls. As in the subepicardial cells, relative mitochondrial volume was not affected by atenolol treatment, but relative sarcoplasmic volume increased almost threefold in the atenolol-treated SHR; an increase in relative sarcoplasmic volume is also evident in the WKY-A group.

It appears, then, that chronic β_1 adrenergic blockade with atenolol had no effect on mitochondrial volume, but attenuated myofibrillar growth and increased the amount of sarcoplasm. These results were consistent in both myocardial layers.

Mitochondrial/Myofibrillar Ratio

Comparison of mitochondrial/myofibrillar (mito/myo) volume ratios in the subepicardium (Fig. 3) indicates that there is no significant difference in this ratio between WKY and SHR control groups. However, the decreased myofibrillar volume seen in treated SHR resulted in a significantly higher mito/myo volume ratio compared to nontreated SHR. In the hypertrophied cells of the subendocardium of SHR mito/myo volume ratio was lower than in WKY ($P < 0.01$). Atenolol treatment normalized the ratio to a value similar to that of WKY. However, treatment of WKY did not significantly alter this ratio in either region of the left ventricle.

DISCUSSION

This study suggests that cardiac β_1 receptors play a major role in the development of cardiac hypertrophy in the spontaneously hypertensive rat. Not only cardiac mass, but also the subcellular composition of the cardiocyte is significantly modified upon treatment with the β_1 adrenoceptor antagonist, atenolol.

Left Ventricular Mass

Several antihypertensive drug regimens have been shown to prevent or reverse cardiac hypertrophy in SHR. Left ventricular mass, measured as absolute weight or as LVW/BW ratio, was modified to approximately the same extent with atenolol as with α-methyldopa (Sen et al., 1974; Tomanek et al., 1979; Sen and Bumpus, 1979; Spech et al., 1980) and with captopril, an angiotensin-converting enzyme inhibitor (Sen et al., 1980; Antonaccio et al., 1979). The data from this study are in agreement with an earlier study by Richer et al. (1978) which also demonstrated a modified LVW/BW ratio in SHR treated with atenolol.

The notion that cardiac hypertrophy is a direct response to pressure overload stimuli in cases of essential hypertension in humans

and in SHR has been contradicted by a number of studies. The vasodilatory action of hydralazine directly upon the smooth muscle cells of the peripheral vasculature successfully prevented or reversed hypertension in SHR in a number of studies (Weiss and Lundgren, 1978; Sen et al., 1974; Antonaccio et al., 1979; Uchiyama et al., 1977; Pfeffer et al., 1977; Weiss et al., 1974), but had no effect on cardiac mass. This paradox has been attributed, in part, to a baroreceptor-mediated increase in cardiac output following administration of the drug. In order to block this reflex, hydralazine was used in combination with the sympathetic blocking agent, guanethidine (Weiss and Lundgren, 1978), or the combination of a blocking agent and diuretic, reserpine, and hydrochlorothiazide (Sen and Bumpus, 1979; Tomanek, 1979b). This use of multiple agents resulted in not only the reduction of blood pressure, but also a moderate reduction in left ventricular mass. However, this approach to modifying left ventricular mass is somewhat limiting in that the factors responsible for cardiac hypertrophy cannot be identified. Inasmuch as single drug treatments may or may not affect a particular parameter, in this case cardiac hypertrophy, cumulative effects of multiple drug regimes may be more complex than their simple additive effects. The dissociation of hypertensive levels of blood pressure and left ventricular hypertrophy in SHR has support from other studies. Neither sympathectomy (Cutilletta et al., 1977) nor adrenal demedullation (Tomanek et al., 1982) nor the two in combination (Tomanek et al., 1982) modifies cardiac hypertrophy despite their moderate effectiveness in modifying blood pressure.

While most β-adrenergic blocking drugs modify the development of hypertension, their effect on cardiac mass in not entirely clear. A slight decrease in left ventricular mass was noted in female, but not male, SHR following treatment with propranolol (Weiss and Lundgren, 1978; Pfeffer et al., 1977) or with timolol (Pfeffer et al., 1977). Cardiac mass was reduced, however, in nonhypertensive rabbits injected with propranolol (Vaughan Williams et al., 1977; Vaughan Williams and Raine, 1974).

Previous documentation of modified cardiac hypertrophy seen following atenolol treatment in SHR has been limited to left ventricular weight (Richer et al., 1978, 1980). While our results are in concert with these studies, our data demonstrate that reduced heart weight is due mainly to alteration of the fiber size increase in the subendocardial region of the left ventricle.

Intracellular Composition

Pressure overload-induced cardiac hypertrophy is characterized by an increased volume of contractile elements and/or a decrease in the volume of energy-producing organelles (Page et al., 1972; Goldstein et al., 1974; Anversa et al., 1976; Lund et al., 1976; Lund and Tomanek, 1978; Tomanek et al., 1979). However, few investigators have studied the effects of antihypertensive drug treatment on intracellular elements. Our results indicate that when hypertrophy in SHR is modified by atenolol the myocytes are characterized by an apparently normal, rather than disproportional growth of myofibrils; we also showed that the diminished growth of myofibrils was associated with a substantial increase in sarcoplasmic volume. That the latter was not due to enhanced water content was verified by the normal water content of left ventricular samples. Since relative mitochondrial volume was not significantly altered by atenolol treatment, it is suggested that the growth of these organelles was proportional to the overall growth in cell width. Thus, it appears that the major intracellular effect of atenolol treatment is attenuation of myofibrillar growth. The observed normalization of the mitochondrial/myofibrillar ratio was due primarily to a decrease in the denominator rather than to an increase in the numerator.

The normalization of intracellular components, like normalization of cell size, is not exclusively linked with blood pressure levels. When SHR were treated with α-methyldopa in doses which did not signficantly affect blood pressure, cardiac hypertrophy was modified and myocardial cell compartment volumes were normalized to WKY values (Tomanek et al., 1979). This could be interpreted as supporting evidence for neuronal regulation of left ventricular mass and organelle growth. Indeed, neonatal sympathectomy with nerve growth factor antiserum has been shown to normalize the mito/myo volume ratio in SHR (Page and Oparil, 1978). This normalization, however, occurred in the presence of fully developed left ventricular hypertrophy, a finding which suggests that cardiac mass and organelle volumes are regulated by separate factors. This contention is

further supported by recent work from our laboratory which demonstrated that sympathectomy and adrenal demedullation had no effect on left ventricular mass in SHR but did prevent myofibril volume from reaching the high values characteristic of untreated SHR and thus facilitated a normal mito/myo volume ratio (Tomanek et al., 1982).

GENERAL COMMENTS

The precise role of β_1 receptors in the development of cardiac hypertrophy is not clear. The ultimate effect of any stimulus which acts via the β_1 receptor to induce cardiac hypertrophy would be dependent upon two factors: 1) receptor number and 2) the receptor's affinity for its ligand. If cardiac hypertrophy is dependent upon an agent stimulating the cardiac β_1 adrenoceptor, then modulation of either receptor number or affinity could act as the regulator. While actual receptor number appears to be decreased in most rat models of hypertension (Woodcock et al., 1978, 1979; Savarese and Berkowitz, 1979; Limas and Limas, 1978), other reports suggest no difference in number (Giachetti et al., 1979) and even an increased number of receptors (Limas, 1979). Most studies agree that while receptor number may be different in the hypertrophied myocardium, affinity of the receptor is unchanged (Woodcock et al., 1978, 1979; Savarese and Berkowitz, 1979; Limas and Limas, 1978).

If a dynamic relationship between receptor occupancy and receptor number truly does exist, as suggested by Glaubinger and Lefkowitz (1977) and supported by other experimental evidence (Glaubinger et al., 1978; Arnett and Davis, 1979; Pik and Wolleman, 1977 Kunos et al., 1978), then obviously this factor must be considered when evaluating β_1 receptor number in the different hypertensive-hypertrophic models. The lower heart rates of treated SHR in our study provide evidence that atenolol was effective as a β_1 antagonist. However, the exact role of β receptors in the development and maintenance of hypertrophy in SHR remains to be defined.

In conclusion, our data implicate myocardial cell β receptors in the type of cardiac hypertrophy characteristic of SHR. On the basis of this study and other recent investigations, it appears that myofibrillar growth is dependent upon β adrenoceptors. However, the role of these receptors in the magnitude of cardiac hypertrophy is less clear. Though atenolol is a β-adrenergic antagonist, the attentuation of left ventricular cardiac hypertrophy could be related to factors which are secondary to β blockade, e.g., decreased heart rate and/or cardiac output (Thomas et al., 1981).

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