Hypertensive Cardiomyopathy
Myocyte Nuclei Hyperplasia in the Mammalian Rat Heart

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Abstract

To determine whether long-term hypertension leads to hyperplasia of myocyte nuclei in the heart, a phenomenon suspected to occur in humans, renal hypertension was produced in rats and the animals were killed 8 mo later. Arterial blood pressure remained elevated for ~ 5 mo, but decreased progressively in the last 3 mo so that at 8 mo this parameter was practically identical to that found in controls. Moreover, left ventricular end diastolic pressure was markedly increased in experimental animals in association with a substantial decrease in left ventricular dP/dt. The alteration of these physiological measurements was indicative of severe ventricular dysfunction. Quantitative analysis of the transmural distribution of myocyte nuclei in the left ventricle showed 36 and 23% increases in myocyte nuclei concentration in the epimyocardium and endomyocardium, respectively. These changes in nuclei were accompanied by 25 and 16% reductions in myocyte cell volume per nucleus in the outer and inner layers of the wall. In conclusion, long-term hypertension leads to impairment of ventricular function and proliferation of nuclei in myocytes. (*J. Clin. Invest.* 1990. 85:994–997.) left ventricle • nuclear proliferation • renal hypertension • cardiac dysfunction

Introduction

It is a general belief that once myocyte proliferation ceases by the age of weaning in the rat, both physiologic and induced myocardial growth occur primarily through hypertrophy of myocytes (1). In contrast, a hyperplastic component persists in interstitial fibroblasts and capillary endothelial cells, which in combination with cellular enlargement (2) participate in the expansion of the nonmyocyte compartment of the myocardium. Moreover, cardiac myocytes differ from those of skeletal muscle and vascular tissue in their potential for cellular responses and growth mechanisms. In skeletal muscle, satellite cells present within the muscle are suspected to be responsible for most of the proliferative process accompanying muscle regeneration (3, 4), whereas in vascular tissue smooth muscle cell hyperplasia appears to take place from dedifferentiation of matured myocytes (5). Although these observations support the concept of the inability of cardiac muscle cells to synthe-

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size DNA shortly after birth (6, 7), myocyte nuclear and cellular hyperplasia have recently been shown in right ventricular hypertrophy of the adult rat heart (8, 9). These unexpected results were regarded as a late event in response to a sustained mechanical stress, and the time factor after the imposition of the overload was considered a critical determinant of the cellular mechanisms implicated in long-standing cardiac hypertrophy (8). Concomitantly, evidence was found that adult rat ventricular myocytes can be stimulated in vitro to synthesize DNA leading to nuclear and cellular proliferation (10). Similar cellular processes have also been shown in vitro (11) and in vivo (12, 13) for atrial myocytes. Because of the prevailing involvement of the left ventricle in heart disease, the hypothesis was tested that the long-term effects of renal hypertension may result in a hyperplastic growth reaction of myocyte nuclei in the left myocardium, raising the possibility that cardiac muscle cells may all resume their capacity to divide compensating for tissue damage and cell loss.

Methods

28 male Fischer 344 rats (Harlan Sprague Dawley, Inc., Indianapolis, IN; NIH colony) at 4 mo of age (body weight, 296±27 g) were used in this study. Two-kidney, one-clip renal hypertension (14) was performed in 18 animals, and 10 sham-operated rats served as controls. Arterial blood pressure was first measured 2 wk after clipping and monthly thereafter using the tail cuff method (14). Hypertension was defined as systolic arterial blood pressure ≥ 150 mmHg. All animals were killed 8 mo after surgery. Body weights at death were 437±26 g in renal clipped animals and 459±25 g in control rats. Care of animals was in accordance with institutional guidelines.

After anesthesia (chloral hydrate, 300 mg/kg i.p.), the right carotid artery was cannulated with a microtip pressure transducer catheter (Millar Instruments, Houston, TX) connected to an ES 2000 chart recorder (Gould Inc., Cleveland, OH). The transducer was then advanced into the left ventricle for the evaluation of ventricular pressures and dP/dt. At the completion of the hemodynamic measurements the hearts were arrested in diastole and fixed by perfusion of the coronary vasculature (15), and the weights of the left and right ventricles were recorded.

30 specimens of the free wall of each left ventricle, extending from the endomyocardial to the epimyocardial surface, were embedded in araldite (15). 10–15 randomly chosen tissue blocks were sectioned at 0.75 μm and stained with toluidine blue. Morphometric sampling at ×1,000 consisted of counting the number of myocyte nuclear profiles, $N(n)$, in a measured area, A, of tissue sections in which muscle fibers were cut transversely. A square, uncompressed tissue area of 5,100 μm^2 was delineated in the microscopic field by an ocular reticle containing 42 sampling points (16). 25 fields were evaluated in the endo- and epimyocardial regions of each ventricle to determine the number of nuclear profiles per unit area of myocardium, $N(n)_A$, and the volume fraction of myocytes, $V(m)_V$, in the tissue (16). These determinations were then combined to yield the numerical density of myocyte nuclear profiles per unit area of myocytes, $N(n)_{A(m)}$, in the two regions of the wall. This was done to obtain a uniform reference point for nuclear counts, eliminating the effects of variations in the interstitium.

Nuclear length, $D_{(n)}$, was evaluated from 85–100 measurements performed at ×1,250 in longitudinally oriented myocytes of each region of each ventricle. 10 blocks were cut, and 8–10 measurements of nuclear length recorded from each ventricular layer from each tissue section (16).

From the estimations of $N(n)_{A(m)}$ and $D_{(n)}$, the regional number of myocyte nuclei per unit volume of myocytes, $N(n)_{V(m)}$, was computed using the equation (16) $N(n)_{V(m)} = N(n)_{A(m)}/D(n)$.

Corresponding myocyte cell volume per nucleus, $V(m)_n$, was obtained by dividing the reference volume of myocytes, $V(m)$, by the respective number of myocyte nuclei: $V(m)_n = V(m)/N(n)_{V(m)}$.

The total number of myocyte nuclei in the ventricle, $N(n)_T$, was derived from the average value per unit volume, $N'(n)_{V(m)}$, and the aggregate myocyte volume in the ventricle: $N(n)_T = N'(n)_{V(m)} \times V(m)_T$.

Morphometric data were collected blindly and the code was broken at the end of the experiment. The magnitude of sampling used in this investigation was selected on the basis of the principle of Poisson statistics (9). By this approach, sampling error for nuclear length in each region of the ventricle in each animal was 10–11%, whereas counting error for nuclear numerical density was 8–10%. The nested analysis of variance (9) performed after the code was broken demonstrated that the number of blocks sampled, the number of nuclei counted, and the number of sampling points used were in excess of what would have been the minimum required for optimum efficiency. Results are expressed as mean±SD. Statistical significance for comparisons between the endo- and epimyocardial regions of each group of animals and between the two animal groups was determined using analysis of variance and the Bonferroni method (9). Significance levels for comparisons between average values across the ventricular wall in the two groups of animals, as well as comparisons of hemodynamics measurements, were evaluated using the unpaired two-tailed t test. Values of $P < 0.05$ were considered to be significant.

Results

Measurements of systolic arterial blood pressure showed that this parameter increased 2 wk after clipping, from 128±12 mmHg in controls to 183±16 mmHg in experimental animals. Blood pressure remained elevated for ~ 5 mo, averaging 180±20 mmHg, but decreased progressively in the last 3 mo so that at death similar levels were found in control (130±14 mmHg) and experimental (132±15 mmHg) rats. Physiological parameters taken just before killing showed that left ventricular end-diastolic pressure was markedly increased, from 6.0±3.1 mmHg in sham-operated rats to 24±12 mmHg in animals with renal artery constriction ($P < 0.001$). A substantial decrease in dP/dt was also evident in experimental rats (control, 12,800±1,900 mmHg/s; experimental, 8,100±1,700 mmHg/s; $P < 0.001$), indicating the presence of left ventricular dysfunction in this group. The impairment of cardiac performance was associated with no change in left ventricular weight (control, 801±50 mg; experimental, 824±52 mg) and right ventricular weight (control, 198±12 mg; experimental, 213±38 mg).

Light microscopic examination of tissue sections of the left ventricle showed that animals with a history of hypertension had multiple areas of replacement and interstitial fibrosis across the wall indicative of focal myocyte cell loss. Because of these pathological changes, the volume fraction of myocytes in the myocardium significantly decreased in the inner (control, 78.7±2; experimental, 70.3±3.4; $P < 0.0001$) and outer (control, 82.5±1.8; experimental, 72.6±3.6; $P < 0.0001$) layers of the ventricle.

Unexpectedly, the lack of hypertrophy in the left ventricle was accompanied by a different number of myocyte nuclear

profiles per unit area of myocytes (Fig. 1 A). With respect to controls, a 39% ($P < 0.0001$) and 50% ($P < 0.0001$) increase in this quantity was measured in the sub-endomyocardial and sub-epimyocardial regions of the wall. These differences resulted in an overall 45% increase from 1,009±62/mm² of myocytes in controls to 1,459±166/mm² of myocytes in experimental animals ($P < 0.0001$).

Myocyte nuclear length is shown next in Fig. 1 B. Renal artery clipping provoked a 10% ($P < 0.0001$) lengthening of the nucleus in epimyocardial myocytes, whereas a 14% ($P < 0.0001$) change was measured in endomyocardial cells. Thus, an overall 12% ($P < 0.0001$) increase was found across the wall. In addition, muscle cells in the epimyocardium possessed nuclei that were 13% ($P < 0.0001$) and 9% ($P < 0.0001$) longer than those of myocytes located in the endomyocardium of control and experimental animals, respectively.

The combination of the changes in myocyte nuclear numerical density and length resulted in a 36% increase in the number of nuclei per cubic millimeter of epimyocardial myocytes ($P < 0.001$) and a 23% augmentation in nuclear concentration per cubic millimeter of endomyocardial myocytes ($P < 0.02$) (Fig. 1 C). These results produced a 30% increase in the average number of myocyte nuclei per cubic millimeter of myocytes across the wall, from a value of 61,012±3,061 in control to a value of 79,240±11,283 in experimental rats ($P < 0.0001$).

At all stages of growth cardiac myocytes of rats are composed of two populations, mononucleate and binucleate cells (17). This phenomenon complicates the quantitative measurement of mean myocyte cell volume (18). Such an analysis, however, can be obtained by introducing the concept of mean cell volume per nucleus, which describes the growth of cellular components on a per nucleus basis. Moreover, this measurement is identical to average cell volume in a mononucleated population of cells.

It was apparent that myocyte nuclei hyperplasia after renal clipping resulted in a 25% ($P < 0.0001$) and 16% ($P < 0.02$) reduction in myocyte cell volume per nucleus in the epi- and endomyocardium, respectively (Fig. 2 A). Average myocyte cell volume per nucleus across the wall decreased by 22% ($P < 0.0001$), from 16,424±819 to 12,870±1,881 μm^3.
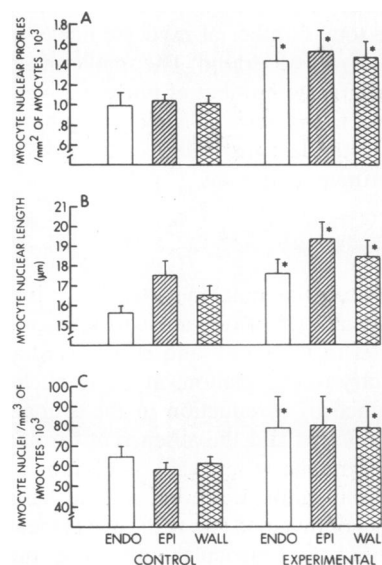

Figure 1. Changes in the number of myocyte nuclear profiles per unit area of myocytes (A), nuclear length (B), and the number of myocyte nuclei per unit volume of myocytes (C) in the endomyocardium (open bars), epimyocardium (hatched bars), and across the ventricular wall (cross-hatched bars) 8 mo after renal hypertension. *, Values statistically significantly different ($P < 0.05$) from corresponding values in control animals.
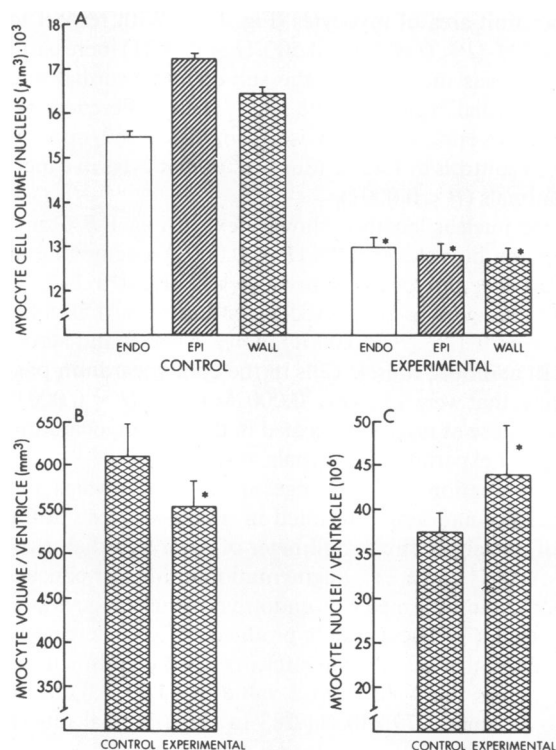
Figure 2. Changes in myocyte cell volume per nucleus in the endo-myocardium (*open bars*), epimyocardium (*hatched bars*), and across the ventricular wall (*cross-hatched bars*) 8 mo after renal hypertension (*A*). *, Values statistically significantly different ($P < 0.05$) from corresponding values in control animals. Changes in the aggregate volume of myocytes in the ventricle (*B*) and in the total number of myocyte nuclei in the entire ventricular wall (*C*) 8 mo after renal hypertension. *, Values statistically significantly different ($P < 0.05$) from corresponding values in control animals.

Fig. 2 shows the aggregate ventricular volume of the myocyte compartment (*B*) and the number of myocyte nuclei in the whole ventricle (*C*). As a consequence of myocardial damage and myocyte loss, a 9% decrease in myocyte mass ($P < 0.005$) was measured in experimental rats, although left ventricular weight was 3% greater in this group. Moreover, an 18% ($P < 0.01$) increase in the total number of myocyte nuclei in the ventricle of hypertensive rats was found. The smaller magnitude of increase in the aggregate number of nuclei per ventricle (Fig. 2 *C*) with respect to that seen on a per unit volume basis (Fig. 1 *C*) was due to the fact that the overall myocyte mass was reduced in experimental animals.

Discussion

The results of this study indicate that renal hypertension in rats leads in 8 mo to left ventricular dysfunction and a hyperplastic response of myocyte nuclei in the endo- and epimyocardial regions of the wall. Contrary to expectation, myocyte nuclei hyperplasia was accompanied by a reduction in the average myocyte cell volume per nucleus and the absence of cardiac hypertrophy in terms of ventricular weight gain or addition of muscle mass. This apparent paradox, however, finds its basis in the fact that multiple foci of myocyte loss with replacement fibrosis were seen throughout the ventricular wall. Thus, the

evolution of hypertensive hypertrophic cardiomyopathy in this animal model seems to be characterized by a first phase of compensatory hypertrophy and little alteration of cardiac performance (19), followed by a second phase in which long-standing hypertension produces severe myocardial injury with marked abnormalities in ventricular hemodynamics. Moreover, the initial stage is fully reversible with restoration of normal contractile behavior (20).

The absence of cardiac hypertrophy at the organ level found 8 mo after renal artery clipping raises the possibility that ventricular weight did not increase during the entire period of study. Although this contention cannot be ruled out, it has been shown that renal hypertension in Fischer rats is associated with ventricular hypertrophy up to 2.5 mo after the onset of hypertension (14). Moreover, the reduction in cardiac mass observed here can not be attributed to poor growth since body weight was reduced by only 5% ($P < 0.05$) at death. Therefore, myocyte loss and replacement fibrosis appear to be the most likely mechanisms of the reduction in ventricular mass in long-term renal hypertension.

In animal experimentation of short-term pressure hypertrophy the myocardial adaptation has been found to consist of cellular enlargement with little or no DNA synthesis in the expanding myocytes (21, 22), so that the possibility of nuclear and cellular hyperplasia has been severely questioned. Studies performed in humans, however, have indicated that when the heart is subjected to a prolonged and sustained load, hyperplasia of myocyte nuclei occurs (23–25). Furthermore, the process of proliferation appears to characterize the phase of transition from compensated physiological hypertrophy to cardiac dysfunction and overt failure (23). The present results are consistent with this sequence of events since myocyte nuclei hyperplasia was found in conjunction with depressed ventricular performance. Thus, the heart is capable of responding to a chronic overload by increasing the number of myocyte nuclei not only in the left ventricle as shown here but also in the right ventricle (8) and both atria (12, 13). Although the pathway by which the initial mechanical message of increased stress on the myocardium is translated by the myocytes into DNA synthesis remains a fundamental unresolved problem, this synthetic process can be triggered in fully mature cells providing an additional, compensatory growth reserve mechanism of the adult heart. This event may constitute the ultimate response of the myocardium to a sustained mechanical stress before intractable ventricular dysfunction supervenes.

Several methodologies have been applied in an attempt to estimate the fundamental processes of myocyte growth, i.e., cellular hypertrophy and cellular hyperplasia. By enzymatic dissociation of myocytes from the ventricular tissue the frequency of myocyte nuclei per cell can be evaluated (17, 26), although average cell volume and cell number cannot be measured. As a result of flattening and spreading of the isolated cells on the surface of a microscope slide, mean myocyte volume is grossly overestimated (27, 28). Moreover, the percentage of myocardium represented by the myocyte population cannot be determined, making the computation of myocyte number impossible. This is an essential parameter that cannot be assumed to remain constant, as clearly shown in this investigation. The estimation of myocyte cell volume by the use of a Coulter counter (Coulter Electronics Inc., Hialeah, FL) is also questionable (28). Such a methodology will yield an average myocyte volume without discriminating between mono- and

binucleated cells, as well as nonmyocyte cells and cell fragments. Finally, the cell isolation process may preferentially preserve one population of cells, leading to an erroneous interpretation of results. So far the quantitative analysis of myocyte nuclei appears to be the only available technique for the study of nuclear hyperplasia in myocardial tissue. The increases in myocyte nuclei numerical density per unit volume of myocytes in the endo- and epimyocardium and in the total ventricle demonstrate that proliferation of myocyte nuclei can be induced in adult left ventricular myocytes. Whether this event is associated with selective survival of mono- or binucleated cells that undergo nuclear division is an important issue which remains to be determined.

Whether nuclear hyperplasia corresponds to an effective increase in cell number cannot be established at present. The proportion of mono- and multinucleated cells in the myocardium is not readily obtainable from quantitative analysis of tissue sections (8, 18), complicating the computation of absolute cell number in the ventricle. Similar limitations also apply to the previous estimations of cell size and number performed in human hearts (23–25). However, growing evidence points to the possibility that nuclear hyperplasia reflects a comparable magnitude of cellular hyperplasia (9). Moreover, in vitro studies clearly demonstrate that myocyte cellular hyperplasia can be induced by different growth factors and 12-0-tetradecanoyl-phorbol-13-acetate (10), supporting the concept that myocyte proliferation may be elicited in the adult heart in vivo.

Acknowledgments

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