Myofilament-Polyribosome Association in Muscle Cells of Rat Left Atrium After Short-Term Hypertension

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SUMMARY Hypertension was induced in uninephrectomized Wistar rats by administration of deoxycorticosterone acetate (DOCA) and by addition of NaCl to their drinking water. The ultrastructure of atrial and ventricular cells of left hearts was compared after short-term (2 and 6 weeks) increased blood pressure. No morphological features could distinguish cells of treated animals from cells of normotensive rats after 2 weeks of treatment. The sarcoplasm of the atrial cells of 6-week-treated hypertensive rats presented an abnormally high number of helical arrangements of ribosomes often associated with abundant unorganized thick filaments, irregular nuclear profiles showing foldings and convolutions, and enlarged mitochondria. The only fine structural changes observed in the ventricular cells of the same animals was a moderate mitochondrial enlargement. The described alterations of atrial cells probably correspond to enhanced synthesis of contractile elements associated with increased nuclear-cytoplasmic exchanges; their absence in ventricular cells suggests that short-term and moderate pressure overload induces adaptive changes in left atrial cells at a stage when ventricular cells have morphological characteristics close to normal cells. (Hypertension 3: 725-729, 1981)

KEY WORDS - atrial cells - heart ultrastructure - DOCA-salt rat - ribosome - myosin filament - atrial cells

ELECTROCARDIOGRAPHIC alterations of the atrial complex in man have been reported in systemic arterial hypertension, even in the absence of definite clinical evidence of left ventricular involvement. We have similarly reported an increased amplitude of the P-wave experimentally in rats following 10 weeks of sustained hypertension produced by DOCA-salt. This finding stimulated us to investigate whether there was a morphological counterpart in the initial stages of increased blood pressure that could lead to the observed electrophysiologic changes of hypertrophy of the left atrium. Moreover, most of the reports on cardiac fine structure in experimental hypertension deal with the study of ventricular alterations. Only a few papers concern the atrial ultrastructure after differently induced heart overload. Our results suggest an early increase in the synthesis of myofilament elements by left atrial cells submitted to moderate pressure overload, a change that appears to precede the observed morphological alterations in left ventricular cells.

Material and Methods

Six Wistar male rats aged 4 weeks were uninephrectomized, and then subcutaneously injected with 12.5 mg of deoxycorticosterone acetate (DOCA) in oil weekly for 5 weeks. Drinking water was replaced by 1% sodium chloride water solution. Their systolic blood pressure (SBP) was measured, under ether anesthesia, every week by the tail plethysmographic method. After 2 weeks of treatment, all the rats showed values over 135 mm Hg. They were divided into two groups of three animals each and sacrificed 2 and 6 weeks later. Rats from the 2-week group had an average SBP of 137.8 mm Hg and weight of 219 g. In the 6-week group, the average SBP was 144.2 mm Hg and weight, 233.3 g. Two groups of 2 male age-matched Wistar rats that received no treatment were used as controls. The average SBP of 137.8 mm Hg and weight of 219 g. In the 6-week group, the average SBP was 144.2 mm Hg and weight, 233.3 g. Two groups of 2 male age-matched Wistar rats that received no treatment were used as controls. The average SBP of 137.8 mm Hg and weight of 219 g. In the 6-week group, the average SBP was 144.2 mm Hg and weight, 233.3 g. Two groups of 2 male age-matched Wistar rats that received no treatment were used as controls. The average SBP of 137.8 mm Hg and weight of 219 g.
Figure 1. Longitudinal sections of ventricular myocytes. Left: Normotensive rat. X 17,400. Right: 6-week-treated hypertensive rat, showing moderate enlargement of mitochondrial profiles. M = mitochondria. X 17,000.

Figure 2. Obliquely sectioned atrial myocytes. Left: Normotensive rat showing no ultrastructural changes of atrial muscle cells. X 10,400. Right: 6-week-treated hypertensive rat showing foldings and convolutions of nuclear membrane and enlarged mitochondria with round or oval shapes. X 9100. N = nucleus; M = mitochondria.
Under ether anesthesia, the thorax of all animals was opened, the lateral walls of the left atrium and of the left ventricle excised and immediately immersed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. Small tissue fragments of the middle layer of the lateral walls were cut and kept for 2 hours in vials containing the same fixative. The tissue blocks were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated in a series of graded ethanol and propylene oxide, and embedded in Epon 812 (Luft). Thick sections (1 μm) were cut on a LKB ultramicrotome III, and stained with alkaline toluidine blue to select artifact-free areas for ultrathin sectioning. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed with a Philips EM 300 electron microscope at 80 kV.

Results
The fine structure of left atrial and ventricular myocytes of rats following 2 weeks of DOCA-saline treatment was similar to the one observed in control animals. After 6 weeks, treated rats presented marked morphological alterations of atrial cells. On the basis of their ultrastructural features, the left atrial muscle cells of this group were clearly more modified by the experimental procedure than the left ventricular cells, the only structural change found in the latter myocytes being a moderate enlargement of the mitochondrial profiles (fig. 1). The atrial cells showed abnormalities in the shape of nuclear profiles, in the content of the sarcoplasmic matrix, as well as enlargement of mitochondria (fig. 2). Nuclei of these cells were frequently irregular due to foldings and convolutions of their limiting membranes.

Accumulations of scattered thick filaments (15-17 nm in diameter) and dense particles (about 20 nm in diameter) were observed in the sarcoplasm of the atrial cells of 6-week-treated hypertensive animals. Dense particles were frequently seen along the filaments, either in a chain-like pattern, or arranged in groups of variable number of elements (from 2 up to 9) in the periphery of the filament network (fig. 3). Associations of thick filaments and dense particles were found in several regions of the sarcoplasmic matrix, such as the sarcoplasmic core, the subsarcolemmal area, or near incomplete myofibrillar bundles elsewhere in the sarcoplasm (fig. 4). They were clearly more frequent and occupied more extensive areas in the sarcoplasmic core. Near the myofibrils were thick filaments in partial association
with dense particles, being incorporated in adjacent sarcomeres by their uncoated portion (fig. 3). Helical arrangements of dense particles, not associated with thick filaments, were also seen in the same sarcoplasmic areas where associations between thick filaments and dense particles were observed (fig. 3).

Discussion

Marked ultrastructural changes were found in the left atrial contractile cells of rats following 6 weeks of sustained arterial hypertension. They consisted of sarcoplasmic accumulation of dense particles associated with thick filaments, isolated helical arrangements of dense particles, irregular nuclei, and enlarged mitochondria. The left ventricular myocytes of the same group of animals appeared to be less affected by the pressure overload, as only a mild enlargement of the mitochondrial profiles could be found in these cells, with no evidence of mitochondrial destruction or of increase in the area occupied by myofibrils. The sarcoplasmic filaments described in the atrial cells are most probably myosin filaments, on the basis of their diameter and of observations suggesting incorporation into sarcomeres. The size of the dense particles and their occasional arrangement in isolated helical groups strongly suggest ribosomes or polyribosomes.

Close associations between thick filaments and ribosomes have been previously described in insect flight muscle, beating heart muscle cells cultured in vitro, regenerating skeletal muscle, Purkinje fibers of several species, and rabbit ventricular myocardium after acute pressure overload induced by thoracic aorta tight stenosis. The myofilament-polyribosome associations described here are similar to the Type V myofilament-polyribosome complex of Thornell's classification, and are widely accepted as corresponding to myosin synthesis in situ by polyribosomes. The helical arrangements of ribosomes may also represent morphological evidence of active protein synthesis as they have been reported in developing muscle cells. It has been shown by radioautographic methods that protein synthesis in striated muscle is preferentially located at the periphery of myofibrils with a subsequent accumulation of the newly formed contractile elements inside the sarcomeres. Assuming that the thick filament-polyribosome associations found in atrial cells of hypertensive rats do correspond to myosin synthesis in situ, we find it interesting that the synthesis of contractile proteins in these cells is located at the same subcellular areas as in ventricular cells. Nevertheless, we did not observe any association between the sarcoplasmic reticulum and newly formed filaments, as proposed by Anversa and coworkers for ventricular cells. Irregular profiles of nuclear membranes have been described in ventricular cells submitted to increased workload and may correspond to an adaptive change that increases the area for nucleocytoplasmic exchanges.

These alterations seem to document an early response of left atrial cells to pressure overload. It is important to point out that in this experimental model of hypertension (providing gradual and moderate increase in blood pressure) our observations indicate that, after short-term hypertension, the left atrial cells undergo cytological transformations suggestive of increased myofibrillar synthesis at a stage at which the left ventricle only presents changes of hyperfunction (mitochondrial enlargement) as defined by Meerson. Further ultrastructural studies comparing the morphology of left atrial and ventricular cells of hypertensive animals are necessary to find out whether atrial cells undergo, as it has been well documented in ventricular cells, more severe alterations eventually leading to degenerative changes after long-term severe hypertension.

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