

Electron Microscopy Studies on the Ultrastructure of the Myocardium in Spontaneously Hypertensive Rats

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A number of morphological studies on the ultrastructure of the myocardium show that myocardial hypertrophy is associated with hypertrophy of the individual cardiomyocytes, as well as an increase in their number (hyperplasia), hyperplasia of the cellular organelles, alterations in cell nuclei and interstitial proliferation. The various subcellular components increase or decrease disproportionately, i.e. can be regulated individually and different patterns may be formed depending on the factor initiating cardiac hypertrophy. Electron microscopy studies of the left ventricle of spontaneously hypertensive rats at the age of 1 month do not show significant differences between them and normotensive control animals. Adult (6-month old) spontaneously hypertensive rats, however, exhibit significant differences, including a higher number of nucleoli, fragmentation of mitochondrial cristae, increase in the myofibril/mitochondria volume ratio, proliferation of rough endoplasmic reticulum and rearrangement of the myofibrils, among others. With the progress of hypertension, the myocardial ultrastructure exhibits signs of hypertrophy, as well as initial degeneration.

Key words: myocardium, electron microscopy, ultrastructure, spontaneously hypertensive rat (SHR)

Introduction

The hypertrophy of the cardiac muscle represents perhaps the most important adaptive mechanism of the heart in response to increased workload [11]. A number of morphological studies on the ultrastructure of the hypertrophied myocardium have been done on both human subjects and experimental animal models [3, 11-16]. These studies showed that myocardial hypertrophy was associated with hypertrophy of the individual cardiomyocytes, as well as an increase in their number (hyperplasia), hyperplasia of the cellular organelles, alterations in cell nuclei and interstitial proliferation [11, 12]. Lund and Tomanek reported an increase in myofibril/cell-volume and a decrease in mitochondria/myofibril-volume ratios in spontaneously hypertensive rats (SHR) [15]. They also noted the presence of double intercalated discs and hypolemmal zones with abundant sarcoplasm rich in small mitochondria, Golgi complexes and free filaments. Several studies have also reported a decrease in the capillary density of the hypertrophied myocardium [9, 15]. Goldstein et al. studied the ultrastructural features of the hypertrophied left ventri-

cle in rabbits [4]. They discovered that the ultrastructure of the mitochondria was normal but their respiratory activity was increased. Furthermore, they observed an abundance of ribosomes and granular endoplasmic reticulum, widening of the Z bands and distortions of the intercalated discs, which were all interpreted as signs of increased protein synthesis. This and other studies concluded that the various subcellular components increase or decrease disproportionately, i.e. can be regulated individually and different patterns may be formed depending on the factor initiating cardiac hypertrophy [1, 4, 11].

Over the course of development of hypertrophy, the myocardium undergoes three distinct stages of changes. The first stage is characterised by an increase in protein synthesis and energy production with preserved cardiac function [16]. A stable state of cardiac hyperfunction exists in the second stage [16]. The third stage is associated with gradual exhaustion of the heart's protein synthesising apparatus, damage of the myofibrils, insufficient renewal of the mitochondria and eventually cellular atrophy [16]. Spontaneously hypertensive rats (SHR) are often used as a reliable model of hypertension in humans, which allows researchers to study the morphological basis and exact mechanisms through which hypertension leads to cardiac hypertrophy [8, 10, 12, 15].

Electron microscopy findings in the cardiomyocytes of young SHR (1-month-old)

Electron microscopy studies of the left ventricle of SHR at this early age do not show significant differences between SHR and control animals of the Wistar strain [12]. Our studies on 1 month old SHR revealed the presence of one or more nuclei, mostly centrally located. The nuclear membrane was clearly visualised and was mostly smooth, with very few convolutions (**Fig. 1**). In line with the increased protein synthesis and growth of the cardiomyocytes associated with this period of the postnatal development, we noted the presence of finely dispersed chromatin and multiple nucleoli in a large number of nuclei. Mitochondria were arranged in one or more rows between the nuclei and the myofibrils and were most numerous in the central sarcoplasmic spaces and the subsarcolemmal sarcoplasm (**Fig. 2**). Cury et al. reported the presence of various shapes of mitochondria: round (with approximately equal major and minor diameter); elongated (with major diameter significantly longer than the minor diameter) and mitochondria with irregular shape [2]. Palmer et al. divided the mitochondria into subsarcolemmal and interfibrillar based on their location [17]. Transmission electron microscopy (TEM)

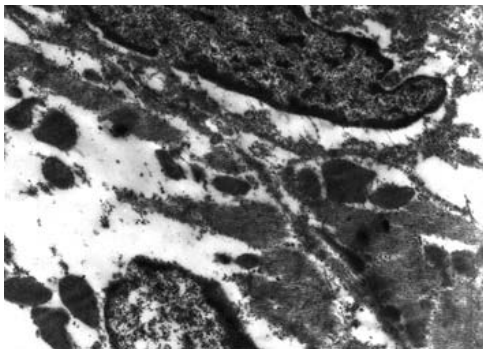


Fig. 1. Electron micrograph of the nucleus of a cardiomyocyte in a 1-month-old spontaneously hypertensive rat (SHR). Magnification x 7000

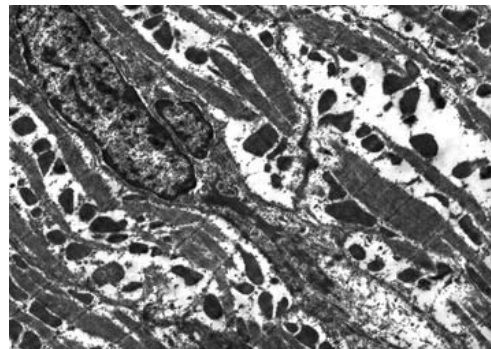


Fig. 2. Electron micrograph showing rows of mitochondria in a cardiomyocyte in a 1-month-old spontaneously hypertensive rat (SHR). Magnification x 4300

also makes it possible to visualise mitochondrial cristae which can be either lamelliform or tubular [7]. Riva et al. discovered that the cristae in subsarcolemmal mitochondria are lamelliform, while those in interfibrillar mitochondria tend to be tubular [18]. Goto et al. studied the ultrastructural characteristics of the sarcoplasmic reticulum and the abundance of caveolae in several groups of SHR and compared them with control animals [5]. These authors found that in young (5-week-old) animals the findings were consistent

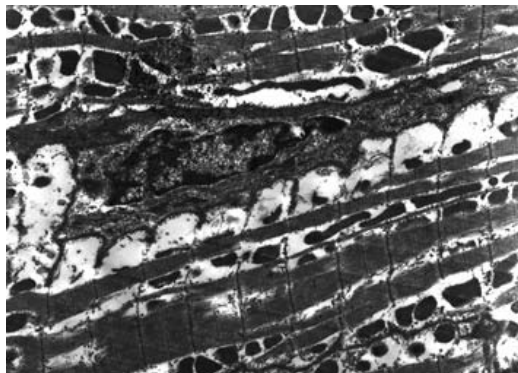


Fig. 3. Electron micrograph showing arrangement of the sarcomeres in a cardiomyocyte in a 1-month-old spontaneously hypertensive rat (SHR). Magnification x 4300

with the normal structure observed in the control group. In our studies on the ultrastructure of the myocardium of SHR, we also observed and described the structure and specific orientation of the myofibrils. They exhibited clearly visible cross-striations, with notable Z, I, A and M bands and were oriented along the long axis of the cardiomyocyte (**Fig. 3**). As described by previous studies [11, 12], at this early age, the sarcomeres exhibit various stages of maturation. In less mature cardiac muscle cells, the thin and thick filaments could be arranged more loosely, with characteristic accumulation of glycogen granules between them [12]. Furthermore, these authors report that less mature and poorly organised sarcomeres tend to be located in the subsarcolemmal zone, in close proximity to abundant elements of rough endoplasmic reticulum, which is consistent with the process of myofibrillogenesis.

Electron microscopy findings in the cardiomyocytes of adult SHR (6-month-old)

In adult (6-month-old) animals, cardiac hypertrophy initiated by the elevated blood pressure reaches its full development. As shown by our previous studies, this process is related to increase in the thickness of the free wall and cross-sectional area of the cardiomyocytes and their nuclei, as well as accumulation of collagen fibres in the subendocardial and interstitial zone [8, 10]. The electron microscopic study of the subcellular structures revealed marked changes from normotensive control animals and the group of young SHR. Kawamura et al. reported that this stage of hypertrophy is associated with heterogeneous changes in the contractile elements, the mitochondria and the sarcoplasmic reticulum, which suggests heterogeneous malfunctions in later stages of cardiac hypertrophy [12]. In our studies, we noted that the nuclei of hypertrophied cells had a bigger number of nucleoli and their membranes were highly convoluted (**Fig. 4**). These findings confirm the data of Maron et al. on human hearts [16] and can be interpreted as enhanced transcription in line with the increased protein synthesis in hypertrophied cells. The ultrastructural organisation showed signs of intracellular oedema. We noted that the mitochondria appeared swollen and there was evidence of fragmentation of the cristae in some of them (**Fig. 5**). Similar results were obtained by Kawamura et al., who also noted disproportional changes in the volumes of the myofibrils and the mitochondria, resulting in a significant increase in the myofibril/mitochondria volume ratio [12]. Goto et al. reported an abnormal rearrangement of the caveolae leading to formation of caveolar conglomerates distributed in bands with variable width, parallel to the long axis of the myocardium in adult animals with significant hypertension [5]. They also

observed a higher density of the rough sarcoplasmic reticulum and fragmentation of the mitochondrial cristae [5]. The organisation of the myofibrils also appears to be altered in adult SHR. Upon comparison with the age-matched control animals and young SHR, we noted that myofibrils in 6-month-old SHR had a much more chaotic distribution, with more prominent Z-bands (**Fig. 6**). Similar findings were reported by other authors [11, 14]. An interesting finding was described by Kawamura et al. who observed that myofibrils from adjacent cells were fixed in different stages of contraction [12]. Myofibrils on one side of the intercalated disc showed pronounced contraction, while on the other side, myofibrils were less markedly contracted. A possible explanation could be dissynchronous contraction in hypertrophied cells of adult animals. The same study found the intercalated discs in older animals tend to be longer and much more convoluted [12]. Legato et al. reported that pressure overloaded hearts contain smaller yet more numerous mitochondria [14]. They also noted the presence of double intercalated discs. Another prominent feature of cardiomyocytes from hypertensive animals was the presence of polyribosomes aligned along the long axes of thick filaments, which could be explained by the increased protein synthesis [14]. Fragments of myofilaments, rough endoplasmic reticulum and polyribosomes were also described under the sarcolemma and in close proximity to the intercalated disc and interpreted as areas of sarcomerogenesis [14].

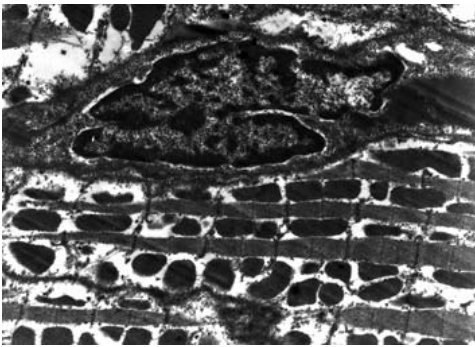


Fig. 4. Electron micrograph of the nucleus of a cardiomyocyte in a 6-month-old spontaneously hypertensive rat (SHR). Magnification x 7000

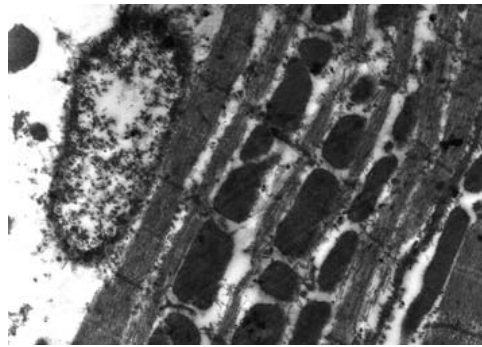


Fig. 5. Electron micrograph showing mitochondria with signs of degeneration in a cardiomyocyte in a 6-month-old spontaneously hypertensive rat (SHR). Magnification x 12 000

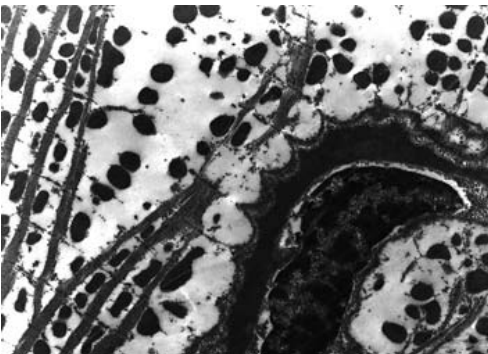


Fig. 6. Electron micrograph showing arrangement of the sarcomeres in a cardiomyocyte in a 6-month-old spontaneously hypertensive rat (SHR). Magnification x 4300

Conclusion

While the ultrastructural organisation in young SHR is mostly consistent to findings in normotensive control animals, adult SHR exhibit marked alterations. Changes in the various subcellular elements are heterogenous but it can be concluded that at the age of 6 months, the myocardial ultrastructure is consistent with the process of hypertrophy and intensive protein synthesis, while also beginning to exhibit signs of initial degeneration.

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