Adaptation of the heart to hypertension is associated with maladaptive gap junction connexin-43 remodelling.

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Summary

We hypothesized that hypertension-related myocardial remodelling characterized by hypertrophy and fibrosis might be accompanied by cell-to-cell gap junction alterations that may account for increased arrhythmogenesis. Intercellular junctions and expression of gap junction protein connexin-43 were analysed in rat heart tissues from both spontaneous (SHR) and L-NAME model of hypertension. Isolated heart preparation was used to examine susceptibility of the heart to lethal ventricular fibrillation induced by low potassium perfusion. Ultrastructure observation revealed enhanced neo-formation of side-to-side type while internalisation of end-to-end type (intercalated disc-related) of gap junctions prevailed in the myocardium of rats suffering from either spontaneously or L-NAME induced hypertension. In parallel, immunolabeling showed increased number of connexin-43 positive gap junctions in lateral cell membrane surfaces, particularly in SHR. Besides, focal lost of immunopositive signal was observed and more frequently in hearts of rats treated with L-NAME. There was a significantly higher incidence of hypokalemia-induced ventricular fibrillation in hypertensive compared to normotensive rat hearts. We conclude that adaptation of the heart to the hypertension-induced mechanical overload results in maladaptive gap junction remodelling that consequently promotes development of fatal arrhythmias.

Key words

Gap junction remodelling - connexin-43 – hypertension - malignant arrhythmias
Introduction

Alteration of structure (remodelling) is fundamental response of the heart to injury or disease. A change in cardiac structure inevitably leads to a change in cardiac function, but the complex relationship between altered structure and function are not fully revealed. Although structural remodelling serves important adaptive purposes, maladaptive consequences of remodelling are likely to contribute to morbidity and mortality in patients with heart disease due to myocardial infarction or systemic hypertension (He and Whelton 1999). Development of cardiomyocyte hypertrophy allows the heart to function better in condition of chronic hypertension, however, structural alterations can also change patterns of electrical activation of the heart that may enhance the risk for malignant arrhythmias, such as ventricular tachycardia and fibrillation. Indeed, conduction slowing and unidirectional conduction block, necessary for initiation and maintenance of re-entrant circuit, often arise in viable, but structurally altered myocardium (Gardner 1985, Peters et al. 1997). We hypothesize that structural alterations may not involve only cardiomyocyte and extracellular matrix changes, but also intercellular junction alterations.

There are three types of intercellular junctions, preferentially located at the intercalated disc, which ensure synchronized electromechanical function of the heart. Adhesive junctions, such as desmosome and fascia adherens, are responsible for cell-to-cell adhesion and cell-to-cell contractile force transduction, intermyocyte electrical coupling and metabolic signal transduction are ensured via gap junctions. These specialized membrane regions contain numerous connexin protein channels, which directly connect the cytoplasmic compartments of two adjacent cells.

Goal of our study was to investigate alterations of myocardial gap junctions, as well as susceptibility of the heart to ventricular fibrillation using two different rat models of experimental hypertension.

Material and Methods.

The investigation conformed to the NIH Guide for the Care and Use of Laboratory Animals. Experiments were performed on male rats that were either spontaneously hypertensive (SHR) or hypertension was induced in 12-week-old Wistar rats by treatment with L-NAME (40mg/kg for 4 weeks, see Pechanova et al. 1999). Males were used, as it is known that they are more sensitive to heart failure and as they
express less connexin-43 compared to females (Soukup et al. 2001, Tribulova et al. 2005). Heart and aorta tissues were excised from sixteen week-old hypertensive and age-matched non-hypertensive rats. Blood pressure was measured using tail-cuff method. Perfusion technique with glutaraldehyde was performed to fix thoracic aorta followed by procedure to obtain slices suitable for quantitative image analysis, as previously described (Kristek and Gerová 1996). Glutaraldehyde fixed ventricular heart tissue samples were routinely processed for transmission electron microscopy examination. Myocardial cryostat sections were used for in situ immunodetection of major gap junction protein, connexin-43 using mouse monoclonal antiCx43 antibody and FITC conjugated goat anti-mouse antibody (details in Tribulova et al. 1999). The hearts from each group (SHR and WKY n=16, L-NAME and Wistar n=12) were perfused in Langendorff mode with oxygenated Krebs-Henseleit solution (KHS) at constant pressure and temperature followed by 15 min perfusion (unless VF occurred earlier) with K⁺-deficient KHS to induce sustained ventricular fibrillation (details in Tribulova et al. 2001). Statistical significance between groups was determined by Student’s t-test or Fisher’s exact test for comparison of the incidence of ventricular fibrillation. The data were expressed as means ± S.E.M. and the differences were considered as significant when p<0.05.

Results.
Compared to non-hypertensive rats, there was a significant increase of blood pressure in SHR and L-NAME-treated animals: 206 ± 5,13 and 180 ± 3,8 vs. control 138 ± 10 (WKY) and 127 ± 2,15 mmHg (Wistar). Hypertension induced cardiac hypertrophy as indicated by increase of heart to body weight ratio from 3.12 ± 0.04 and 2.68 ± 0.03 to 4.32 ± 0.33 and 3.06 ± 0.16, as well as by apparent thoracic aorta remodelling (Table 1). The latter was documented by significant increase of arterial wall thickness in both hypertensive models compared to normotensive rats.

Development of VF due to perfusion of the heart with low K⁺ solution preceded ventricular premature beats and transient arrhythmias that occurred earlier in hypertensive rat hearts (not shown). Independent on the etiology, hypertensive rat hearts were significantly more vulnerable to develop sustained VF, during 15 min of low K⁺ perfusion, compared to non-hypertensive rats (Fig.1). Accordingly, sustained VF occurred in five of eight SHR hearts and in five of six L-NAME-treated rat hearts, while only in two WKY or Wistar rat hearts.
Electron microscopic examination revealed heterogeneous population of cardiomyocytes in the left ventricles of both groups of hypertensive rats. Characteristic changes are documented in Fig. 2 (L-NAME model). Besides normal (Fig. 2A), numerous cardiomyocytes hypertrophied, possessing active nuclei, enhanced rough sarcoplasmic reticulum, increased number of mitochondria, new myofilaments and creation of a lateral adhesive junctions (Fig. 2B). The latter was followed by neoformation of side-to-side type of gap junctions (Fig. 3B). On the other hand, some hypertrophied cardiomyocytes exhibited degenerative subcellular changes consisting of electron lucent oedematous mitochondria, focal myocytolysis and non-uniform sarcomere shortening (Fig. 2C). In addition, enhanced accumulation of collagen and fibrosis were observed in L-NAME model only (not shown).

Hypertension-induced subcellular changes were accompanied with cell-to-cell junctions alterations, which were heterogeneously distributed throughout myocardium. As shown in Fig. 3 (SHR model) alterations were characterized by dehiscence (separation) of fascia adherens junctions (Fig. 3A), by neoformation of side-to-side gap junctions (Fig. 3B) and by internalisation of end-to-end (intercalated disc-related) type of gap junctions (Fig. 3C).

In parallel with these changes immunolabeling of connexin-43 revealed two features of alterations that were present in both models of hypertensive rats. There was an enhanced expression confined to lateral site-to-site type of gap junctions (Fig. 4B) and focally diminished or lost intercalated discs-related connexin-43 expression (Fig. 4C). The former feature was more pronounced in SHR while the latter in L-NAME-treated rat hearts.

Discussion.

Many observations suggest that the myocardial architecture as well as the number, size and spatial distribution of gap junctions play an important role in determining the conduction properties of cardiac tissues (Spach and Heidlage 1995). Reduction in the total amount of gap junction profile length as well as gap junction protein expression in diseased ventricular myocardium have been implicated in the pathogenesis of slow conduction and unidirectional conduction block leading to re-entrant arrhythmias (Peters et al. 1997, Saffitz et al. 1999, Severs 2001).
In agreement with it, results of this study likewise our previous studies suggest that chronic or acute impairment of intercellular coupling at the gap junctions precedes occurrence of malignant arrhythmias. (Tribulova et al. 2001, Tribulova et al. 2002).

Changes in gap junction distribution (remodelling of gap junctions) usually results from chronic pathophysiological stimuli (e.g. hypertension, diabetes, ischemia), acute pathophysiological conditions (e.g. hypokalemia, ischemia/ reperfusion) induce temporary alterations of gap junctions indicated by marked reduction or lost of connexin-43 immunopositivity (Tribulova et al. 2002). While lateralisation of gap junctions decreases myocardial electrical stability likely due to changes in anisotropic conduction (Spach and Heidlage 1995), the acute impairment of electrical coupling can trigger malignant arrhythmia due to re-entrant (re-excitation) mechanism.

It should be noted that hearts exhibiting gap junction remodelling are more vulnerable to lethal arrhythmias also because they are prone to develop Ca$^{2+}$ overload (during acute ischemia or electrolyte disbalance) since they have abnormal Ca$^{2+}$ handling. Ca$^{2+}$ overload can lead to both Ca$^{2+}$ oscillations that can trigger early or delayed after-depolarization and connexin channels inhibition that can induce cell-to-cell uncoupling (de Mello 1986). Indeed, Ca$^{2+}$ overload and cell-to-cell uncoupling were detected previously in hypertensive rat hearts subjected to low potassium perfusion (Tribulova et al. 2001, Tribulova et al. 2002). The former was indicated when cardiomyocytes exhibited contraction bands or nonuniform sarcomere shortening and the latter when relaxed cardiomyocyte was “connected” by gap junctions with contracted one (Fig. 2C) or when severely injured cardiomyocyte was “connected” with slightly injured or even intact one.

In conclusion, there is no doubt that abnormalities of gap junctions may be involved in the development of malignant arrhythmias. On the other hand, because gap junctions are highly dynamic structures they are promising target aimed to prevent or attenuate incidence of lethal events in patients with diseased hearts.

**Acknowledgements**

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References.


Legends.

Fig. 1. Incidence of sustained ventricular fibrillation (SVF) that was induced by perfusion of the heart with low $K^+$ solution for 15 min, unless SVF occurred earlier. SHR – spontaneously hypertensive rats (n=8); W-H hypertensive rats due to L-NAME treatment (n=6); WKY (n=8) and W – Wistar (n=6) age-matched non-hypertensive controls. *P<0.05, hypertensive vs. nonhypertensive.

Fig. 2. Representative pictures showing heterogeneous population of cardiomyocytes in the hypertensive rats. A. Ultrastructure of cardiomyocytes and their junctions are not apparently altered. Note electron dense mitochondria, relaxed sarcomeres and maintained integrity of intercalated disc-related intercellular junctions. B. Hypertrophied cardiomyocytes exhibiting abundant polyribozomes and creating adhesive junctions at the cell-to-cell contacts that precedes gap junction formation. C. Injured cardiomyocytes show electron lucent mitochondria, contracted (*) or relaxed (**) sarcomeres and impaired integrity of intercellular junctions, fascia adherens and gap junction. Arrows – gap junctions, R – polyribozomes, M - mitochondria, FA – fascia adherens junctions, D – desmosomes (adhesive junctions). Bar scale 1 µm.

Fig. 3. Typical subcellular alterations of intercellular junctions found in the hypertensive rat heart. A. Dehiscence (separation) of fascia adherens junctions (FA) and internalisation of gap junction (arrow). B. Neoformation of lateral, side-to-side type of gap junctions between adjacent hypertrophied cardiomyocytes (arrows). C. Internalisation of gap junctions and presence of annular profile (arrow), it precedes their degradation. Bar scale 1 µm.

Fig. 4. Representative pictures showing distribution gap junctions in hypertensive rat hearts immunolabeled with anti-connexin-43 antibody. A. Normal appearance with predominant labelling of intercalated discs-related gap junctions. B. Arrows indicate abundant side-to-side type of gap junctions. C. Decreased density of gap junctions and focal lost of immunofluorescence signal (asterisks). Lines indicate direction of myofibrils. Magnif. 80x.
**Table 1.** Geometry of the thoracic aorta (W – normotensive Wistar rats, W-H – hypertensive rat due to L-NAME treatment, SHR – spontaneously hypertensive rats)

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>W - H</th>
<th>SHR</th>
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<tbody>
<tr>
<td>Wall thickness (µm)</td>
<td>63.16±2.37</td>
<td>81.87±1.69*</td>
<td>81.08±2.27*</td>
</tr>
<tr>
<td>Cross sectional area (µm²) x10³</td>
<td>343±11.6</td>
<td>483±9.78*</td>
<td>452±18.35*</td>
</tr>
<tr>
<td>Inner diameter (µm)</td>
<td>1690±44.37</td>
<td>1792±32.78</td>
<td>1688±36.81</td>
</tr>
<tr>
<td>Wall thickness/inner diameter x10²</td>
<td>3.78±0.19</td>
<td>4.55±0.13*</td>
<td>4.81±0.17*</td>
</tr>
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* P<0.01 hypertensive vs. normotensive Wistar rats
Fig. 1.
Fig. 4.