Regulation of myocardial connexins during hypertrophic remodelling

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Cardiac hypertrophic remodelling, initiated by signalling cascades in response to increased workload, injury or intrinsic disease, is initially adaptive. However, prolonged hypertrophy as a consequence of pathological stress leads to maladaptive changes that increase the risk for fatal ventricular arrhythmias. One of these changes is the remodelling of myocardial gap junctions, which provide for electrical coupling of adjacent cardiomyocytes. Myocardial gap junctions are composed of three connexin isotypes, connexin40 (Cx40), -43 (Cx43), and -45 (Cx45) and each display a characteristic developmental and regional expression pattern. Alterations in the distribution and expression of Cx43, the predominant isoform in the adult ventricles, has been the main focus of examination in humans, experimental animal models and cultured cardiomyocytes in response to hypertrophy. The molecular mechanisms and signalling pathways underlying these changes have been studied less thoroughly. In this review we summarize what is known about the remodelling of myocardial gap junctions during hypertrophy, the putative underlying mechanisms and functional consequences thereof.

Introduction

Cardiac hypertrophy is characterised by an increase in cardiomyocyte size, enhanced protein synthesis and a higher sarcomere organization, which are all preceded and accompanied by a reactivation of several foetal genes. This structural remodelling process of the myocardium is initiated by signalling cascades in response to increased workload, injury or intrinsic disease. It has been suggested that the structural remodelling serves as an adaptive response to sustain normal wall tension and cardiac output.1–4 Specifically in athletes, it is accepted that this exercise-induced hypertrophic response is compensatory. However, prolonged hypertrophy in response to pathological situations inevitably leads to maladaptive changes, increasing the risk for arrhythmias or the progression to heart failure. Recently, it has been proposed that there is a clear distinction between compensatory and maladaptive hypertrophy, of which only the latter leads to heart failure.3 In exercise-induced hypertrophy, the myocardium usually does not exceed a modest increase in ventricular wall thickness and fibrosis is absent. At present it is not clear whether pathologic-maladaptive hypertrophy is first preceded by compensatory hypertrophy and becomes maladaptive when it is prolonged or whether it is entirely maladaptive from the offset. Insight into the underlying molecular mechanisms of...
hypertrophic remodelling may clarify which changes in cellular signalling molecules lead to maladaptive hypertrophy.1–4

A characteristic consequence of hypertrophic remodelling as a result of pathological stress is the increased risk for fatal ventricular arrhythmias. Alterations in action potential (AP) duration, a disturbed Ca\(^{2+}\) metabolism, and ventricular re-entry circuits arising from regions of slow, inhomogeneous conduction, and conduction block may contribute to arrhythmogenesis. The observed alterations in cell-to-cell conduction of electrical impulses may be due to changes in the expression pattern and composition of the gap junctions. Gap junctions are mainly located in the intercalated discs of cardiomyocytes and consist of multiple gap junction channels. A gap junction channel is built of gap junction proteins (connexins); six connexins interact to form a connexon (hemichannel) on one cell surface, which aligns head-to-head with a connexon on the apposing cell surface together forming an intercellular channel.\(^5,6\) In cardiomyocytes, the products of three connexin genes, connxin43 (Cx43), -40 (Cx40), and -45 (Cx45), are expressed. The predominant isofrom in the ventricles is Cx43, which is expressed at very high levels in all working myocytes and, depending on the species, in the whole (human) or distal (mouse and rat) part of the conduction system.\(^7–11\) Both Cx40 and Cx45 are present throughout the ventricular conduction system.\(^7,12–15\) However, for Cx45 very low trace amounts have also been detected in focal parts of the ventricular wall.\(^13\)

A derangement in the intercellular electrical coupling and expression and distribution of connexins may have profound effects on cardiac electrophysiology and arrhythmogenesis. Disruption of the side-to-side cell connections in hypertrophy by interstitial fibrosis, for instance, has been shown to impair cellular impulse propagation.\(^16,17\) Moreover, genetic ablation studies in mice for Cx40 and Cx43 revealed that a reduction of their expression in the heart correlated with an impaired conduction and a higher propensity for arrhythmias.\(^18–22\) Alterations in the amount and distribution of gap junction channels have been frequently correlated with cardiac disease.\(^23–32\) It has been suggested that during the initial phase of hypertrophy, connexins are up-regulated and that they become down-regulated once the hypertrophy becomes prolonged and turns into heart failure.\(^31,32\) However, little is known about the exact mechanisms and signalling pathways regulating connexin expression during hypertrophic remodelling. Insight into these processes may contribute to a better understanding of cardiac diseases that promote arrhythmogenesis and lead to specific targets for novel therapies that prevent the creation of anatomic substrates of lethal ventricular arrhythmias in patients with heart disease.

In this review, we summarize changes in myocardial connexin expression during hypertrophic remodelling, in humans, several animal models and in stimulated cardiomyocytes, and discuss the putative underlying regulating mechanisms and functional consequences.

Connexin expression during hypertrophic remodelling

Myocardial connexin expression in the development of hypertrophy and heart failure has been examined in both humans and animal models. Studies on cardiac tissue of patients with hypertrophic heart disease have been most relevant in correlating connexin remodelling with the cardiac disease process, however they provide only limited information on the regulatory mechanisms involved due to the large variety of disease causes. Experimental animal model studies, in which volume- or pressure-overload has been used as a single pathological stimulus for the initiation of hypertrophy, add to our understanding of connexin remodelling. Primary cultures of (neonatal) cardiomyocytes have also been used to study the effect of mechanical stress on cardiac remodelling.\(^33–41\) An overview of the studies describing connexin remodelling (mainly Cx43) during hypertrophy in humans, animal models and cultured cardiomyocytes is given in Tables 1–3, respectively.

Connexin expression in hypertrophic and failing human hearts

Several studies have described changes in the number, size and distribution of myocardial gap junctions during human hypertrophic heart disease (Table 1). Kostin et al. recently reported that in the left ventricles of pressure-overloaded human hearts with valvular aortic stenosis, Cx43 expression was increased in the compensated hypertrophic stage, but decreased and heterogeneous distributed throughout the ventricles in the decompensated stage.\(^23\) In patients with dilated (DCM), ischaemic (ICM), or inflammatory (MYO) cardiomyopathy it was noticed that there was a redistribution of Cx43 from the intercalated discs to the lateral surfaces (lateralization) of left ventricular myocytes in areas of structural damage. However, in myocardium distant from the structural lesions, the distribution of Cx43 was normal. In the entire left ventricle of all failing hearts, the amount of Cx43 per myocyte was significantly lower than in normal healthy hearts.\(^24\) A decrease in expression\(^27,29\) and/or heterogeneous distribution\(^27\) of Cx43 during hypertrophic heart disease has also been described by others. Moreover, the decreased expression of Cx43 at the protein level is accompanied by a reduction of Cx43 mRNA, suggesting that the down-regulation of Cx43 in hypertrophic heart disease is regulated at the transcriptional level.\(^27\) A decrease in expression and heterogeneous distribution of Cx43 was correlated with ventricular arrhythmias in patients suffering from non-ischaemic dilated cardiomyopathy.\(^26\)

Besides a reduction and redistribution in Cx43, changes in Cx40 and Cx45 have also been reported. Dupont et al. showed that the decline in Cx43 protein and mRNA was accompanied by an up-regulation of Cx40 mRNA and protein at the endocardial surface next to the Purkinje fibres in patients with ICM.\(^27\) This Cx40 up-regulating response was suggested to be compensatory...
for the loss of Cx43. Cx45 mRNA expression, in contrast, appeared to follow the same pattern as Cx43 whereas Cx45 protein was not detectable. In a recent paper of Yamada et al. it was shown that, in conjunction with a down-regulation of Cx43, Cx45 protein, but not Cx45 mRNA, was significantly increased in failing human hearts. This enhanced expression of Cx45 seems to occur in a heterogeneous pattern and is co-localized with Cx43. Heterotypic gap junction channels of Cx43 and Cx45 have a decreased conductance leading to an increased chance for the generation of ventricular arrhythmias. Up-regulation of Cx45 at the protein and not at the mRNA level suggests that post-translational mechanisms are involved in the regulation of Cx45 in heart failure.

Thus, in human maladaptive hypertrophy disturbances in the expression of all three myocardial connexins have been observed, although the mechanisms eliciting these changes appear to be different.

**Connexin expression in hypertrophic animal models**

Several different animal models, in which cardiac hypertrophy was induced by either volume-overload or pressure-overload have been used to study the effect on connexin expression (Table 2). Volume-overload causes eccentric hypertrophy, which is characterised by lengthening of the individual myocytes (with addition of new sarcomeres in series) resulting in ventricular chamber dilatation. An increased risk for ventricular arrhythmias has been seen in diseases (e.g., mitral and aortic valve regurgitation) causing eccentric hypertrophy. Also Cx43 expression appears to be altered during hypertrophic remodelling as a consequence of volume-overload. In an experimental pig model of left ventricular volume-overload by creation of an aorta-v. cava fistula, it was shown that Cx43 expression initially increased during the acute (compensated) hypertrophic response but decreased with the progression of hypertrophy (168 h to 3 months). In contrast, in rabbits with aortic valve regurgitation, causing compensated eccentric hypertrophy, Cx43 expression was less than the age-matched controls at 1 month after surgery, but tended to increase reaching supernormal levels in the chronic (≥2 years), compensated aortic regurgitation animals. In another rabbit model of volume-overload, in which an arteriovenous shunt was made between the common carotid artery and jugular vein,
Cx43 mRNA expression was significantly depressed relative to sham-operated animals 12 weeks after the shunt formation. Treatment with an angiotensin II type 1 receptor antagonist during week 6–12 after shunt formation tended to restore Cx43 mRNA expression as compared to animals treated similarly with vehicle (saline) alone. Although this antagonist-induced restoration of Cx43 mRNA expression was statistically non-significant, it still
suggests that angiotensin II is involved in the regulation of Cx43 expression during hypertrophic remodelling as a consequence of volume-overload.

A variety of Cx43 alterations have also been reported in animal models of concentric hypertrophy, which arises as an adaptation to pressure-overload. Concentric hypertrophy is characterised by a lateral increase in myocyte size (with addition of new sarcomeres in parallel) and thickening of the myocardial walls without a dilatation in chamber-lumen.\(^{3,4,42}\) In guinea pigs with surgical aortic stenosis it was shown that during the progression from LV hypertrophy to congestive left heart failure, there was substantial intercalated disc remodelling, including a significant decrease in the amount of Cx43 per LV myocyte in the failing LV (6 months after surgery). No alteration in Cx43 was observed at the compensated hypertrophy stage (4 weeks after surgery).\(^{48}\) On the other hand, both in a rat model of right ventricular (RV) hypertrophy secondary to monocrotaline (MCT)-induced pulmonary hypertension and a rat model of LV hypertrophy as a result of abdominal aortic banding, a dispersion of Cx43 over the entire cell surface and a proportional decrease of Cx43 at the intercalated disc centres was observed.\(^{49,50}\) This remodelling of Cx43 was apparent 2 weeks after MCT injection and 8 weeks after aortic banding.\(^{49,50}\) It remains unclear whether the remodelling of Cx43 in these studies marks a transition from compensated hypertrophy to maladaptive hypertrophy (heart failure). In MCT-induced rats it was shown that the RV hypertrophy was accompanied by altered anisotropic conduction properties in the RV, likely caused by the dispersed expression of Cx43. Blocking angiotensin II signalling, by administration of the angiotensin II receptor antagonist losartan to the aortic-banded rats, reduced Cx43 disorganization and LV (concentric) hypertrophy.\(^{50}\) This is similar to the effect observed in the volume-overloaded rabbits, suggesting that angiotensin II signalling plays either a direct or indirect role in Cx43 remodelling during hypertrophy. In contrast, smooth muscle Cx43, but not cardiac Cx43, was up-regulated at the 4-week stage in two rat models, in which hypertension and eventually cardiac and aortic hypertension was induced by constriction of the left renal artery or by left nephrectomy followed by DOCA-Salt administration.\(^{51}\) No signs of cardiac failure were reported in these two hypertrophic rat models.

In the above described volume- and pressure-overload animal models, changes in myocardial connexins other than Cx43 were not studied, except for the chronic aortic stenosis guinea pig model, in which it was reported that there is no compensatory up-regulation of Cx45 when Cx43 is decreased.\(^{48}\)

In general, Cx43 expression appears to be unaltered or up-regulated during the initial and compensatory phase of hypertrophy, but redistributed along the cardiomyocyte surface and reduced when the hypertrophy becomes prolonged and putatively maladaptive (in its progression to heart failure). Nevertheless, there is some heterogeneity in Cx43 remodelling data that may be caused by the different experimental procedures used to induce hypertrophy and the different time points studied after the induction. The alterations in Cx43 distribution and expression likely take place as a secondary characteristic to overall alterations in hypertrophic remodelling. For instance, ventricular dysynchrony, that is a disturbed LV mechanical coordination, is a feature frequently observed in heart failure. In a dog model of dysynchronous failing hearts, the lateral endocardium displayed a 60% reduction in Cx43 protein expression relative to neighbouring segments, in contrast with synchronous failing hearts in which there was minimal regional variability in expression.\(^{52}\) This regional heterogeneity in Cx43 expression suggests that local biochemical input is more important in the regulation of Cx43 than systemic haemodynamics or neurohumoral signalling. For instance, the local decrease in Cx43 was accompanied by a 2-fold increase in the expression of the mitogen-activated protein kinase (MAPK) p-erk (ERK), which has been implicated before in negatively regulating Cx43 expression in liver epithelial cells.\(^{52,53}\)

**Mechanical stress-induced connexin remodelling in cardiomyocytes**

Stretching cultured cardiomyocytes resembles the mechanical stress response of the in vivo heart to haemodynamic overload, i.e., enhanced protein synthesis and a reactivation of several foetal genes.\(^{34-36}\) Different directions and degrees of in vitro stretch have been demonstrated to induce cardiomyocyte hypertrophy.\(^{33}\) Stretch models are suitable to study initial cardiac responses, e.g alterations in myocardial connexins, to overload. In a model of 10% linear pulsatile stretch of neonatal rat ventricular myocytes, Cx43 protein appeared to be dramatically up-regulated starting after only 1 h and further increasing after 6 h. This increase in Cx43 expression corresponded to an increase in conduction velocity, and appears to be mediated by vascular endothelial growth factor (VEGF). The same amount of nonpulsatile (static) stretch caused qualitatively similar but quantitatively smaller alterations.\(^{37,40}\) Cyclic mechanical pulsatile stretch (20%) also induces a dramatic increase in Cx43 protein expression.\(^{38,39}\) This enhanced Cx43 protein expression was accompanied by an increase in Cx43 mRNA, suggesting the involvement of transcriptional regulation in Cx43 up-regulation.\(^{38,39}\) However, an enhanced protein stability may also add to this, as the Cx43 protein increase is prolonged despite an earlier decline of Cx43 mRNA.\(^{38}\) Cx40 mRNA expression did not change in response to cyclical mechanical stretch.\(^{38}\)

In a recently developed model of 10%-5% anisotropic static stretch, Cx43 was significantly up-regulated when the highest strain (10%) was applied transverse to the myofibrils.\(^{41}\) Application of the principal strain parallel to the myofibrils had little effect on Cx43 expression. Also the general hypertrophic response appeared to be much more pronounced with the main stretch transverse to the myofibrils. These differential responses may represent the regional heterogeneous pattern of myocyte hypertrophy and suggest that different signalling cascades are associated.
with longitudinal versus transverse stretch. However, at present, both the mechanism behind this and how this affects connexin regulation is not known.

**Signalling pathways potentially involved in Cx43 remodelling**

From the Connexin expression during hypertrophic remodelling, it becomes increasingly clear that alterations in Cx43 are correlated to the stage of hypertrophy. Local changes in signalling molecules, either initial in response to overload or secondary in response to overall remodelling characteristics, potentially trigger the alterations in Cx43 expression. Hypertrophic signalling is a highly integrated process, in which multiple pathways converge and crosstalk. Several mediators of hypertrophic signalling pathways have been implicated in the regulation of Cx43 (summarized in Fig. 1), either by the generation of transgenic mice models (Table 4) or by treating cultured neonatal cardiomyocytes with known inducers of specific hypertrophic pathways (Table 5). Although the response of cultured (neonatal) cardiomyocytes to certain stimulations may sometimes be different than that of the intact, adult heart, these cultures have been very valuable in dissecting the involvement of individual signalling components in the generation of the hypertrophic response in general and in connexin regulation in particular. Nevertheless, it should be emphasised that a comprehensive picture of regulatory factors involved in regulation of connexin expression should come from both in vivo and in vitro studies.

**Transgenic mice models**

Experimental mice models, in which the expression level of a single component of a hypertrophic signalling pathway has been genetically altered, add to our understanding of the underlying pathways of Cx43 remodelling (Table 4).

In a transgenic hypertrophic mouse model of forced retinoic acid signalling, Cx43 was redistributed followed by a significant, heterogeneous reduction of expression in the left ventricle. In severely affected mice, a re-expression of Cx40 was observed in those areas in which Cx43 had almost completely disappeared. The gap junction remodelling was accompanied by a reduction in conduction velocity, however no spontaneous or inducible ventricular arrhythmias were observed. The signal transduction route connecting overexpression of the retinoic acid receptor with alterations in connexin expression is currently not known.

In Connexin expression in hypertrophic animal models, ERK (mitogen-activated protein kinase p-erk) was suggested to be involved in local decreases in Cx43 expression in dysynchronous failing dog hearts.
(c-Jun N-terminal kinase), another MAPK, has also been reported to be involved in the regulation of Cx43. Conditional transgenic JNK mice exhibited a significant reduction in myocardial Cx43 mRNA and protein. However, these mice did not show ventricular hypertrophy, chamber dilatation or fibrosis. It is quite possible that activation of JNK is not enough to function as a forward regulator of hypertrophy, although this does not mean that there is no putative role for JNK in hypertrophic remodelling. Whether JNK is a direct regulator of Cx43 during hypertrophic remodelling and if so, what the underlying mechanism is, still needs to be determined.

JNK has been reported to crosstalk with the calcineurin-NFAT pathway. Calcineurin, a calcium-dependent phosphatase, plays both a sufficient and indispensable role in the development of cardiac hypertrophy, independent of the underlying pathophysiological stimulus. Upon activation, calcineurin dephosphorylates the transcription factor NFAT, allowing its translocation into the nucleus where, together with other transcription factors, it induces gene expression. Transgenic mice over expressing a constitutively active form of calcineurin develop severe hypertrophy culminating in heart failure. Cx43 protein is markedly down-regulated, dephosphorylated and redistributed (from the intercalated discs to the lateral borders) within the hearts of these transgenic mice. However, whether calcineurin affects Cx43 expression and processing directly or indirectly and whether NFAT activation is involved is presently not known. Calreticulin, a key upstream regulator of calcineurin, transgenic mice have also been reported to have dramatically reduced levels of Cx43 protein and RNA, together with negligible levels of the phosphorylated form of Cx43. Thus calreticulin, calcineurin and JNK are interrelated and involved in the regulation of Cx43, although presumably this is only part of a more comprehensive and integrated signalling network mediating the alterations in Cx43 during hypertrophic remodelling.

Myotrophin is a 12 kDa soluble protein involved in both the initiation of hypertrophy and the transition to heart failure. Transgenic mice with cardiac-specific overexpression of myotrophin display a reduction in Cx43 mRNA expression at 4 weeks of age, with an even further down-regulation at the HF stage (9 months).

### Table 4 Cx43 expression in transgenic mice models

<table>
<thead>
<tr>
<th>Model</th>
<th>Cx43 protein</th>
<th>Lateralization</th>
<th>Heterogeneity</th>
<th>Cx43 mRNA</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic DCM mice by forced retinoic acid signalling</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Re-expression of Cx40 in severely affected mice. Reduction of conduction velocity</td>
<td>54,55</td>
</tr>
<tr>
<td>Transgenic HF mice by overexpression of activated calcineurin</td>
<td>↓</td>
<td>+</td>
<td></td>
<td></td>
<td>Also marked dephosphorylation of Cx43</td>
<td>62</td>
</tr>
<tr>
<td>Transgenic HF mice by overexpression of myotrophin</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td></td>
<td>Decrease of Cx43 RNA at onset of cardiac hypertrophy (4 weeks), with an extra down-regulation at the HF stage (9 months)</td>
<td>65</td>
</tr>
<tr>
<td>Transgenic HF mice by overexpression of Nkx2.5</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td></td>
<td>Mice died before 4 months of age of HF associated with conduction abnormalities</td>
<td>70</td>
</tr>
</tbody>
</table>

Abbreviations: DCM, dilated cardiomyopathy; HF, heart failure; ↑, up-regulated; ↓, down-regulated; –, unaltered; +, yes; –, no. Lateralization refers to a redistribution of Cx43 from the intercalated discs to the lateral surfaces of the myocytes. Heterogeneity indicates a heterogeneous distribution of Cx43 throughout the ventricles.

### Table 5 Cx43 expression after treating NCM with biochemical inducers of hypertrophy

<table>
<thead>
<tr>
<th>Induction of NCM</th>
<th>Cx43 protein</th>
<th>Cx43 RNA</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II 24 h</td>
<td>↑</td>
<td>↑</td>
<td>Increase in Cx43 protein was blocked by adding losartan</td>
<td>71</td>
</tr>
<tr>
<td>Dibutyryl-cAMP 24 h</td>
<td>↑</td>
<td>↑</td>
<td>Increase of Cx45 protein but not mRNA. Increase in conduction velocity</td>
<td>72</td>
</tr>
<tr>
<td>JNK overexpression</td>
<td>↓</td>
<td>↓</td>
<td>JNK overexpression also decreases Cx43 protein and mRNA in vivo</td>
<td>56,80</td>
</tr>
<tr>
<td>Myotrophin 24 h</td>
<td>↑</td>
<td>↑</td>
<td>Also a 2-fold drop in Cx43 proximal promoter activity</td>
<td>70,73</td>
</tr>
<tr>
<td>Nkx2.5 overexpression</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NCM, neonatal cardiomyocytes; JNK, c-Jun N-terminal kinase; ↑, up-regulated; ↓, down-regulated; –, unaltered.
 Alterations in connexin protein and RNA levels during the hypertrophic response are often correlated, suggesting that transcriptional regulation is involved. Cx43 expression is affected by Nkx2.5, a transcription factor which has been demonstrated to be up-regulated in hypertrophied hearts.67–69 In transgenic mouse hearts over expressing wild type Nkx2.5, which eventually results in four-chamber enlargement and heart failure, a dramatic reduction in Cx43 protein and RNA expression has been reported.70 Although direct evidence is lacking, the above results suggest that Nkx2.5 could be one of the mediators of Cx43 down-regulation during hypertrophic heart disease. However, since Nkx2.5 interacts with other cardiac transcription factors involved in hypertrophic remodelling,67 more transcription factors are presumably involved in the regulation of Cx43.

Biochemical inducers of connexin remodelling in cardiomyocytes

The effects of hypertrophic stimuli,71 signal transduction molecules,56,72 and transcription factors,70,73 known to be involved in hypertrophic signalling, on myocardial connexin expression have been investigated in cultured neonatal cardiomyocytes (Table 5).

Angiotensin II has already been shown to be involved in Cx43 down-regulation during in vivo hypertrophy (see Connexin expression in hypertrophic animal models56,58), since treatment with an angiotensin II type 1 receptor antagonist tended to restore the Cx43 expression. In contrast with this, during in vitro stretch (Mechanical stress-induced connexion remodelling in cardiomyocytes39), the observed Cx43 up-regulation was blocked by adding the angiotensin II type 1 receptor antagonist losartan. In addition, stretch significantly increased the angiotensin II concentration in the heart during hypertrophic remodelling.71 The molecular mechanism behind this apparent discrepancy still needs to be elucidated. Most likely additional, post-transcriptional mechanisms are involved in JNK-mediated down-regulation of Cx43 as well, because of the near 90% reduction in protein compared with the 40% reduction in mRNA. As stated in Transgenic mice models, further research is necessary to determine whether JNK directly influences Cx43 expression and, if so, what the mechanism is, or whether it possibly influences Cx43 via crosstalk with other signalling routes, such as the calcineurin pathway.

In contrast with the observed in vivo down-regulation of Cx43 mRNA in myotrophin transgenic hearts, in vitro treatment of cultured neonatal cardiomyocytes for 24 h with myotrophin showed a 4-fold increase in Cx43 mRNA.80 Similar to angiotensin II, these conflicting results could be due to the cellular context (whole heart versus cultured neonatal cardiomyocytes), however, the way in which overexpression was achieved (expression from transgenic gene versus extracellular addition of myotrophin protein) could also contribute to this.

The involvement of Nkx2.5 in the regulation of Cx43 has also been determined in cultured neonatal ventricular cardiomyocytes. Similar to the hearts of the Nkx2.5 transgenic mice (Transgenic mice models), neonatal cardiomyocytes, in which Nkx2.5 is over expressed by adenoviral infection, display dramatically reduced levels of Cx43 protein and mRNA.70,73 In addition, Nkx2.5 over expression also elicited an approximately 2-fold drop in Cx43 proximal promoter activity, although this does not fully explain the severe drop in Cx43 mRNA. Presumably, additional transcription factors, repressor sites or other molecular mechanisms are necessary for the down-regulation of Cx43 in neonatal cardiomyocytes. Whether and how Nkx2.5 overexpression during in vivo hypertrophy affects the Cx43 promoter still has to be determined.
**Functional consequences of connexin remodelling**

There has been some discussion about the functional consequences of altered expression of Cx43 during hypertrophic remodelling. Genetic ablation studies demonstrated that low levels of Cx43 are an important determinant in the development of ventricular arrhythmias in mice. In these cases, however, ventricular Cx43 content was diminished to less than 10% of control. In maladaptive hypertrophy, Cx43 content decreases to only 40–60% of control values. By using a simple computer-simulation model of strands of human ventricular myocytes, it was found that a 40–60% reduction in junctional conductance gives rise to only moderate changes in conduction velocity and anisotropy ratio. In these calculations, cell size was kept constant. When cell size is increased by 50%, as is often the case in hypertrophic hearts, reduction of longitudinal conduction velocity remains moderate, while in the transverse direction conduction velocity even increases by about 20%. However, the used model did not incorporate heterogeneity in gap junction reduction throughout the ventricle. In addition, other disease-induced changes such as fibrosis or changes in action potential configuration were not included. In a pathological heart, Cx43 expression is heterogeneous, i.e., some regions have a virtually normal density of Cx43 expression while others lack Cx43 almost completely. In conjunction with fibrosis and other disease-related changes, this gives rise to discontinuities in conduction which may be sufficient to generate an arrhythmogenic substrate. In mice engineered to express Cx43 heterogeneously such behaviour was indeed demonstrated. Also mechanical performance of these so-called chimeric hearts was jeopardized, which suggests that mechanical asynchrony of the left ventricle may be related to heterogeneity of Cx43 expression among other factors.

Therefore, controlled intervention in the molecular pathways underlying hypertrophy-induced connexin remodelling may eventually reduce the risk for lethal ventricular arrhythmias and improve the mechanical performance of diseased hearts.

**Perspectives**

Examinations of Cx43 expression in hypertrophic hearts obtained from humans and animal models have shown that both its level of expression and distribution pattern are frequently altered relative to corresponding control hearts. The stage of hypertrophy appears to be important for the type of change that occurs: Cx43 expression is unaltered or up-regulated during the compensatory phase of hypertrophy, but redistributed along the cardiomyocyte surface and reduced when hypertrophy is prolonged and progresses to heart failure. It is anticipated that animal model studies for cardiac hypertrophy and failure, in which Cx43 expression is followed over time, will further substantiate this concept. In vitro studies with primary neonatal cardiomyocytes, in which hypertrophy was induced biochemically or by genetically altering the expression level of specific signalling molecules, have provided molecular factors involved in Cx43 up- and down-regulation (see Fig. 1). Such in vitro studies will provide further information on signal transduction pathways involved in the regulation of Cx43 expression both in general and during the hypertrophic response. Targeting these in vitro determined pathways in animal models of hypertrophy and heart failure will undoubtedly clarify their role in vivo. Although the frequently found correlation between alterations in Cx43 protein and RNA expression suggests the involvement of transcriptional regulation, the distribution and degree of Cx43 expression in response to hypertrophic signalling is most likely determined at more than one level. Besides transcriptional regulation, changes in translation, trafficking and proteolysis during hypertrophy, can also potentially contribute to an altered Cx43 expression pattern. Protein phosphorylation status of either Cx43 itself or associated regulatory proteins may also contribute to the observed functional differences as well. It is expected that genetic and biochemical/pharmacological studies targeting each of these processes will clarify their role in connexin remodelling and provide new avenues for intervention.

Eventually, the ultimate goal of many research efforts will be to prevent the remodelling of Cx43 expression, which possibly reduces the risk of lethal arrhythmias in compromised hearts. As may be appreciated from this review, it will take considerable time before it is actually reached and manipulation of Cx43 expression becomes a tool in cardiological practice.

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