Gap Junctions in Cardiovascular Disease

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Abstract—Connexins, the protein molecules forming gap junction channels, are reduced in number or redistributed from intercalated disks to lateral cell borders in a variety of cardiac diseases. This “gap junction remodeling” is considered to be arrhythmogenic. Using a simple model of human ventricular myocardium, we found that quantitative remodeling data extracted from the literature gave rise to only small to moderate changes in conduction velocity and the anisotropy ratio. Especially for longitudinal conduction, cytoplasmic resistivity (and thus cellular geometry) is much more important than commonly realized. None of the remodeling data gave rise to slow conduction on the order of a few centimeters per second. (Circ Res. 2000;86:1193-1197.)

Key Words: connexins ■ conduction velocity ■ arrhythmia ■ computer simulation

Gap junctions, specialized membrane structures consisting of arrays of intercellular channels, connect adjacent cells in many tissues and organs, thereby providing chemical and electrical communication. In the heart, gap junctions provide the pathways for intercellular current flow, enabling coordinated action potential propagation. Recently, numerous reports have been published suggesting that changes in gap junction distribution, density, and properties may be involved in the initiation and persistence of various cardiac arrhythmias. In the present review, we summarize the data presented in these reports and discuss functional implications.

Structure and Properties of Gap Junction Channels

In the past decade, the structure and properties of gap junction channels have been extensively documented, as discussed in several recent reviews.1–4 Mammalian gap junction channels are built of connexins encoded by a family of closely related genes. All connexins consist of 4 highly conserved $\alpha$-helical membrane-spanning segments separated by 2 extracellular and 1 intracellular loop. The amino and carboxy terminals are located intracellularly. Fifteen members of the mammalian connexin family have been identified. They differ mainly in the sequence of their intracellular loops and carboxy terminals.

One gap junction channel is formed by head-to-head docking of 2 hemichannels (connexons), each composed of 6 connexin molecules hexagonally arranged around an aqueous pore. Because docking is mediated by relatively conserved extracellular loops, many connexons composed of one kind of connexin can combine with connexons made of other connexins to form heterotypic gap junction channels. A connexon may also be composed of different connexins (heteromeric connexon). In the heart, different connexins colocalize in gap junction plaques, but it is unknown whether heterotypic and/or heteromeric gap junction channels exist in the cardiovascular system.

Gap junction channels are permeable to substances with a molecular weight of $\leq 1$ kDa. Permeability depends on connexin type and charge of the permeating molecule. Gap junction channels behave as gated ion channels. In cardiomyocytes, single-channel conductances range from $\approx 20$ pS for homotypic Cx45 channels to 75 pS for Cx43 channels and to $\approx 200$ pS for Cx40 channels. Gap junction conductance is modulated by transjunctional voltage, by $[H^+]_i$ and $[Ca^{2+}]_i$, by the phosphorylation state of the connexins, and by extracellular fatty acid composition.

Connexin expression is also modulated. Hormones can upregulate or downregulate connexin content. In neonatal rat heart cells in vitro, cAMP can dramatically upregulate Cx43 expression with a concomitant increase in conduction velocity of the action potential. The turnover of connexins is remarkably fast. In the adult rat heart, for example, the half-life is 1.3 hours.

Gap Junction Distribution in Normal Myocardium

In myocardium, connexins are regionally expressed: Cx43 is found throughout the heart, with the possible exception of the nodal tissues and parts of the conduction system.5 In mammalian species, Cx40 is expressed in atrial tissue (with the exception of the rat heart) and in the proximal conduction system (with the exception of the guinea pig heart).6,7 Cx45 expression seems to be limited to nodal tissues and the
conduction system, but some reports claim a much more widespread distribution, probably because of the use of a not completely specific anti-Cx45 antibody. No other connexins have been detected between cardiomyocytes to date.

In adult ventricles, gap junctions exclusively contain Cx43 and are located predominantly in the intercalated disk (ID) region between cells. The anisotropic conductive properties of ventricular myocardium are dependent on the geometry of the interconnected cells and the number, size, and location of the gap junction plaques between them. Many (immuno)-histochemical and (electron)microscopic studies have addressed these issues. Gap junction plaques consist of arrays of more or less closely packed 9- to 10-nm particles, representing individual channels. Under normal conditions, rat gap junction plaques appear to contain ~15% particle-free space, whereas rabbit gap junction plaques are contiguous. In terms of junctional conductance, there is little difference, because the lower number of channels per square micrometer is largely offset by the decrease in access resistance (see below). Mean gap junction plaque area ranges from 0.21 μm² in the human ventricle to ~0.45 μm² in the rat ventricle and to ~4 μm² in the canine ventricle. In the latter case, this area was ~1.5 μm² for approximately half of the plaques and ~6.6 μm² for the other half. From (electron)microscopic and (immuno)histochemical assessment, it appears that larger gap junction plaques are located in interpicate regions of the ID (ie, in regions running more or less parallel with the long axis of the cells) and that smaller ones are located in plicate regions. Hoyt et al estimated 80% of total gap junction area per cell to be located in interpicate regions, where the gap junctions can serve both longitudinal and transverse conduction.

Ventricular myocytes are connected by IDs to ~10 neighbor cells. Conduction velocity is determined by gap junction plaque area in each of these IDs. Total gap junction plaque area per ID is 47 to 94 μm² in rats, 42 or 13.6 μm² in dogs, and ~10 μm² in humans.

In the atrium, gap junction plaques contain both Cx43 and Cx40. Most often, Cx43 and Cx40 are localized in the same plaques without preferential location of either connexin in lateral cell borders or ID plaques. No data are available to calculate the gap junction plaque area per ID in the atrium.

In ventricular myocardium, expression of Cx40 is limited to the conduction system. In most mammalian species, no Cx43 is present in the proximal part (His bundle, bundle branches), whereas in the more distal regions of the bundle branches and the Purkinje fibers, Cx40 and Cx43 are coexpressed. In Xenopus oocytes, Cx43 and Cx40 cannot form functional heterotypic gap junction channels, and it was suggested that the connexin distribution in the proximal conduction system would serve to propagate the action potential rapidly to distal parts without current loss via gap junctions to the surrounding septal myocytes. However, in mammalian cells, the incompatibility of Cx40 and Cx43 connexons seems less clear-cut. In mouse hearts, Cx45 is expressed throughout the atrioventricular node, His bundle, and bundle branches. The expression of Cx40 is limited to the core of the His bundle and bundle branches.

**Distribution of Gap Junctions in Diseased Myocardium**

In virtually all cardiac diseases predisposing to arrhythmias, changes in the distribution and number of gap junctions (gap junction remodeling) have been reported. In advanced ischemic disease, a narrow zone consisting of ~5 layers of cells bordering healed myocardial infarctions was detected. In this zone, the normal distribution of gap junctions in end-to-end–located IDs was disrupted with a shift of Cx43-containing spots to the lateral cell borders without changes in spot size. Normal, ischemic, and hypertrophied human left ventricles showed equally sized plaques of anti-Cx43 staining, but the total amount of Cx43 was reduced by 40% in the diseased hearts. The number of IDs per cell was not different in normal and diseased hearts, which implies that cellular geometry had not dramatically changed. On the other hand, in reversibly ischemic and hibernating human ventricle, a reduction of Cx43 plaque size of 23% and 33%, respectively, was observed in the affected regions, with no changes in normal myocardium. In these experiments, a shift of Cx43 spots from an end-to-end location to a lateral location was observed; this shift was also reported in hypertrophic cardiomyopathy.

In a guinea pig model of congestive heart failure, an overall reduction of Cx43 of 37% was observed at the congestive heart failure stage after 6 months of aortic banding, whereas at the compensated hypertrophy stage, no changes were seen. Recently, a 35% decrease in gap junction area per ID was reported after 4 weeks of right ventricular hypertrophy caused by monocrotaline-induced pulmonary hypertension in the rat, concomitant with a 30% decrease in longitudinal conduction velocity (Θ₁). At the same time, numerous Cx43-positive spots appeared along the lateral borders of the cells. Conduction velocity in the transverse direction (Θ₂) and total Cx43 content, as judged by immunoblotting, were not affected. The authors concluded that the redistribution of gap junction plaques may explain the reduced anisotropy ratio (Θ₁/Θ₂). However, the observed 55% increase in cell diameter may also play a role. Peters et al demonstrated a close correlation between the inducibility of figure-of-8 reentrant arrhythmias in epicardial tissue bordering 4-day-old infarcts in canine ventricles and the disruption of gap junction distribution. Especially viable cells close to and sometimes interdigitating with necrotic cells of the infarcted region showed extensive Cx43 labeling of lateral cell borders.

It appears that localization of gap junctions is a prominent feature of diseased myocardium. It is not completely clear, however, to what extent this localization can contribute to altered conduction properties, because it was recently shown that in rat ventricular cells bordering healed infarcts, many of the lateral gap junction plaques are located in invaginations of the sarcolemma into the cell interior, thereby not contributing to cell-to-cell communication. A comparable observation has been made in right ventricular hypertrophy.

Although quantitative data are scarce, another common finding in diseased myocardium is a 30% to 40% reduction of gap junction area per ID. In (post)ischemic ventricles, this reduction is limited to a few cell layers around the affected area, whereas in hypertrophied ventricles, the reduction is...
more widespread. From this observation alone or together with an increased density of lateral gap junction plaques, one would predict a reduced anisotropy ratio. In one study, an increased anisotropy ratio has been suggested to occur in infarct border zones. This increase was partly due to a reduction in lateral (interpalate) gap junction density and partly to a decrease in the number of cells having side-to-side connections with neighboring cells.

Changes in gap junction density and distribution in diseased atrial tissue are less well documented. In rapidly paced dog atria, an increase in Cx43-positive spots was reported, especially at lateral cell borders. In goat atrium, no apparent changes in Cx43 density and distribution after 16 weeks of sustained atrial fibrillation (AF) were found, although some dephosphorylation had occurred. Cx40 protein was absent in 0.15- to 0.6-mm patches of atrial tissue after 16 weeks of AF without a reduction in Cx40 mRNA. The patchy reduction of Cx40 protein was evident after 2 weeks of AF, around the same time that AF became sustained. Whether this reduction in Cx40 is causally related to the persistence of AF remains to be determined.

Data on the involvement of gap junction remodeling in arrhythmias originating in the conduction system or in nodal tissue are just beginning to be reported.

**Gap Junctions and Conduction Velocity**

**Effective gj**

We carried out some simple computer simulations to test the effects of changes in density and distribution of gap junctions on conduction velocity of the action potential. First, we determined the effective gap junctional conductance (gj, corrected for cytoplasmic access resistance) by use of our previously published model and by assuming the Cx43 single-channel conductance to be 75 pS at 37°C and all channels to be in their conducting state (but see Reference 42). Figure 1A shows that the effects of cytoplasmic access resistance are already apparent for relatively small gap junctions and become more prominent with increasing gap junction size. For gap junctions >0.5 μm², the effective conductance is <50% of the value obtained by simply adding up the individual conductances of all channels in a given area (uncorrected conductance), and for gap junctions >4 μm², effective conductance is even <20%. Consequently, gj per unit surface area is not constant but decreases with increasing gap junction size (Figure 1B). Effective conductance is 0.3 to 0.5 μS/μm² for small to moderately sized gap junctions (0.3 to 1.5 μm²) and <0.2 μS/μm² for large gap junctions (>5 μm²). As discussed above, gap junctional surface area commonly ranges between 10 and 40 μm² per ID. If an average effective conductance of 0.3 μS/μm² is used, effective gj per ID between neighboring cells is 3 to 12 μS.

**Conduction Velocity**

Next, we assessed the importance of gj for conduction velocity. We stimulated the leftmost cell in a linear strand of 50 cells at a frequency of 1 Hz and computed the conduction velocity across the middle third of the strand. Cells were either arranged end to end or side by side, and neighboring cells were connected through a constant (effective) gj (Figure 2A). We used the human ventricular cell model of Priebe and Beuckelmann in a numerical representation of the cable equation similar to that explored by Shaw and Rudy, with a value of 150 Ω cm for cytoplasmic resistivity.

To obtain ΘL values of ~70 cm/s as reported for human ventricles, a conductance of 7.0 μS is required (Figure 2B). This agrees well with the above value of 3 to 12 μS estimated from morphometric data. The same conductance of 7.0 μS results in ΘT of 30 cm/s. Under normal conditions, ΘT is quite insensitive to changes in gj; it decreases by only 9 cm/s (13%) upon halving the conductance. ΘT is more sensitive to changes in gj; it decreases by 36% upon halving the conductance. The relative insensitivity of ΘT to changes in gj can be explained in terms of gap junctional resistivity, which we computed from gj and cell dimensions (Figure 2C). For longitudinal conduction (solid line with filled circles in Figure 2C), gap junctional resistivity falls below the cytoplasmic resistivity of 150 Ω cm (horizontal dotted line) at gj values as small as 2.5 μS, whereas for transverse conduction, gap junctional resistivity is much larger than cytoplasmic.
resistivity at all values of \( g \) (dashed line with open squares). Thus, we conclude that conduction velocity, particularly \( \Theta_L \), is only moderately sensitive to changes in effective \( g \). In addition, cell dimensions (ratio of cell length to cell width) may play a major role in determining the (anisotropy of) conduction velocity. This is consistent with the conclusion of Spach et al.\(^{17} \) that cell size may be more important than gap junction distribution.

**Functional Implications**

The simulation results constitute important caveats for the interpretation of quantitative data from (immuno)histochemical or (electron)microscopic studies. A reduction in total gap junction content by as much as 40% without changes in the size of the gap junction plaques, as observed in diseased human hearts,\(^{26} \) may by itself have only moderate effects on conduction velocity. If normal \( g_L \) between cells is 5 \( \mu S \), a 40% reduction to 3 \( \mu S \) results in an 11% decrease in \( \Theta_L \) from 65 to 58 cm/s and a 27% decrease in \( \Theta_T \) from 24 to 18 cm/s (Figure 2B). The associated anisotropy ratio increases by 22% from 2.7 to 3.3. In other cases, overall gap junction content remained unchanged, but a shift to lateral cell borders occurred.\(^{25,32} \) A 40% shift would reduce \( \Theta_L \) by 11% and increase \( \Theta_T \) by 25%, resulting in a 29% decrease in the anisotropy ratio. If the new lateral gap junctions are located intracellularly,\(^{32,34} \) \( \Theta_T \) may not change at all.

**Concluding Remarks**

In many studies involving the remodeling of gap junctions in diseased ventricular tissue, the authors have concluded that a decrease in conduction velocity may enhance the propensity to reentrant arrhythmias. However, the present analysis of the limited data available indicates that the reduction in conduction velocity or the changes in anisotropy ratio may actually be moderate. Certainly, the changes observed would not bring the substrate in the realm of slow conduction as addressed in several recent experimental\(^{48–50} \) and theoretical\(^{44,51} \) studies. Our analysis indicates that cytoplasmic resistivity and cellular geometry are much more important than commonly realized, a conclusion also advocated by Spach and colleagues.\(^{15,47,52,53} \)

We did not incorporate disease-induced changes in membrane ionic and gap junction channel properties in our analysis. Undoubtedly, such pathophysiological changes further complicate the understanding of arrhythmogenesis in acute ischemia, chronic myocardial infarction, hypertrophy, and heart failure.

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**References**

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