Role of Gap Junctions in Cardiac Conduction and Development

Comments by Dr. Andrés R. Pérez Riera about

Lo CW. Role of gap junctions in cardiac conduction and development: insights from the connexin knockout mice. Circ Res. 2000 Sep 1;87(5):346-8.

Gap junctions are membrane channels that mediate the cell-to-cell movement of ions and small metabolites. In the heart, gap junctions play an important role in impulse conduction. Studies over the last decade have revealed that gap junctions are encoded by a multigene family known as the connexins. There are at least 15 connexin genes in the vertebrate genome.[1] Connexins have been referred to by 2 nomenclature systems, either according to the molecular weight of the protein or by its class as determined by protein sequence.[1] [2] The molecular weight designation will be used here in conformity with the study by Kirchhoff et al[3] in this issue of *Circulation Research*.

In the heart, there are 3 major connexin isotypes expressed: connexin (Cx)43 ($\alpha_1$connexin), Cx45 (α_6 connexin), and Cx40 (α_5 connexin). Each of these connexins exhibits different channel properties and is regulated by different gating mechanisms. Cx43 is the only connexin known to be expressed in the adult working myocardium,[4] although a recent report suggests that Cx37 (α_6 connexin) also may be present.[5] In the specialized conductive tissue of the heart, Cx45 is found in the atrioventricular node and adjoining His bundles.[6] This connexin isotype forms voltage-sensitive channels with very low conductance.[7] In contrast, Cx40, which generates channels with high conductance,[8] is expressed in the fast-conducting tissues of the His-Purkinje system, being nested within the Cx45 expression domain (Figure⇓).[9] [10] In the Purkinje

fibers of the peripheral conductive tissue, Cx43 is coexpressed with Cx40 (Figure⇓).[9] It is hypothesized that this compartmentalized expression of connexins may provide for the orderly and sequential spread of activation from the atrial to ventricular chamber.[9 10 11] Thus, expression of Cx45 in the atrioventricular node and His bundle may electrically insulate the central conductive tissues from the working myocardium. In contrast, Cx40 may allow rapid impulse propagation to distal parts of the conduction system, where Cx43 may additionally facilitate coupling to the surrounding working myocytes.

With the availability of the embryonic stem cell technology for targeted gene disruption, the analysis of the of role of gap junctions in cardiac conduction is now focused on the cardiac phenotype of the respective connexin knockout animal models. The first model to be made was the Cx43 knockout mouse,[12] which dies neonatally from pulmonary outflow obstruction. These mice surviving to term was understandable, because 2 other connexins, Cx45 and Cx40, are expressed in the embryonic and fetal myocardium.[13 14] Nevertheless, the phenotype of this mouse was puzzling, because Cx43 is not known to be expressed in the outflow tract. Recent studies suggest that this cardiac phenotype may involve cardiac neural crest perturbation,[15 16] a migratory cell population that plays an important role in outflow tract tissue remodeling.[17] The neonatal lethality of the Cx43 knockout mouse has precluded the use of electrocardiography to examine cardiac function. However, cardiac function in the viable heterozygous Cx43 knockout adult animals was examined by electrocardiography and optical mapping of surface conduction velocities. Such studies revealed no significant change in conduction velocity, activation pattern, or any other cardiac conduction parameters.[18] These results were confirmed in the study by Kirchhoff et al.[3] Nevertheless, slowing in the spread of

activation was reported in an earlier study of the heterozygous Cx43 knockout mouse.[19] An open question is whether a 50% reduction in Cx43 protein would be expected to have a significant impact on conduction velocity. Surprisingly, recent modeling studies by Jongsma and Wilders[20] suggest that the answer is perhaps not to the degree one might expect. Their computer simulations indicated that even a 40% reduction in gap junction content had only modest effects on conduction velocity. Instead, cell geometry and cytoplasmic resistivity are likely to have greater roles in determining cardiac conduction velocity.[21]

More recently, a Cx40 knockout mouse model was generated by 2 independent groups,[22 23] and although these mice are homozygous viable, they show slowed conduction and a partial atrioventricular block.[21 22] Arrhythmia was also observed, but this was found by only 1 of the 2 groups.[21 22] This discrepancy was attributed to strain background differences,[21 22] a reasonable possibility given that the 2 studies reported QT_{max} intervals for the wild-type littermates that were markedly different. In the study by Kirchhoff et al,[3] a more detailed characterization of the Cx40 knockout mouse model is described. Also presented is a characterization of the cardiac phenotype of the Cx43/Cx40 double-knockout mice. The main conclusion reported is that Cx43 and Cx40 have additive effects on ventricular conduction and heart morphogenesis. Ventricular conduction was examined by electrocardiography, which revealed a very small increase in QRS and QT_{max} intervals in the double-heterozygous knockout mice ($Cx43^{+/-}/Cx40^{+/-}$) as compared with wild-type ($Cx43^{+/+}/Cx40^{+/+}$) or single-heterozygous knockout animals ($Cx43^{+/-}/Cx40^{+/+}$; $Cx43^{+/+}/Cx40^{+/-}$). These changes were proposed to arise from the combined effects of slowed conduction in the central conduction system (where Cx40 is expressed) and in the working myocytes (where Cx43 is expressed).

Surprisingly, the present study also reported that some of the homozygous Cx40 knockout animals died prenatally or neonatally. Thus, half of the homozygous Cx40 knockout mice apparently died between embryonic days 12.5 and 13.5, and a few also succumbed shortly after birth or as young adults. Histological analysis of embryonic day 12.5 hearts revealed no obvious cardiac defects except for incomplete formation of the mesenchymal cap of the atrial septum. The authors suggest that prenatal lethality is likely attributable to functional, not structural, deficits in the heart. However, because Cx40 is expressed in many other tissues besides the heart, including the vasculature, the cause of prenatal lethality in the Cx40-deficient mice remains an open question. Their morphological analysis of a few newborn and adult homozygous Cx40 knockout mice revealed more substantive cardiac abnormalities, including myocardial hypertrophy, common atrioventricular junction, or, in one case, ventricular septal defects. When the Cx40 and Cx43 knockout mice were intercrossed, surprisingly, no $Cx40^{-/-}/Cx43^{+/-}$mice were obtained. Such mice apparently died neonatally. These animals exhibited cardiac septation defects similar to those seen in the homozygous Cx40 knockout mice but more severe. However, in contrast to Cx43 haploinsufficiency, which worsened the Cx40 knockout heart phenotype, $Cx40^{+/-}/Cx43^{-/-}$ littermates were reported to have the same cardiac defects typically seen in the $Cx40^{+/+}/Cx43^{-/-}$animals, which consisted of malformations involving the conotruncus.[12]

These observations led the authors to conclude that Cx43 and Cx40 have additive effects on heart septation. How this might be generated is unclear, because presently there is no known role for Cx43 or Cx40 in heart septation. Also puzzling is that the additive effects of Cx43/Cx40 deficiencies on heart septation are nonreciprocal. Perhaps the septation defects may represent

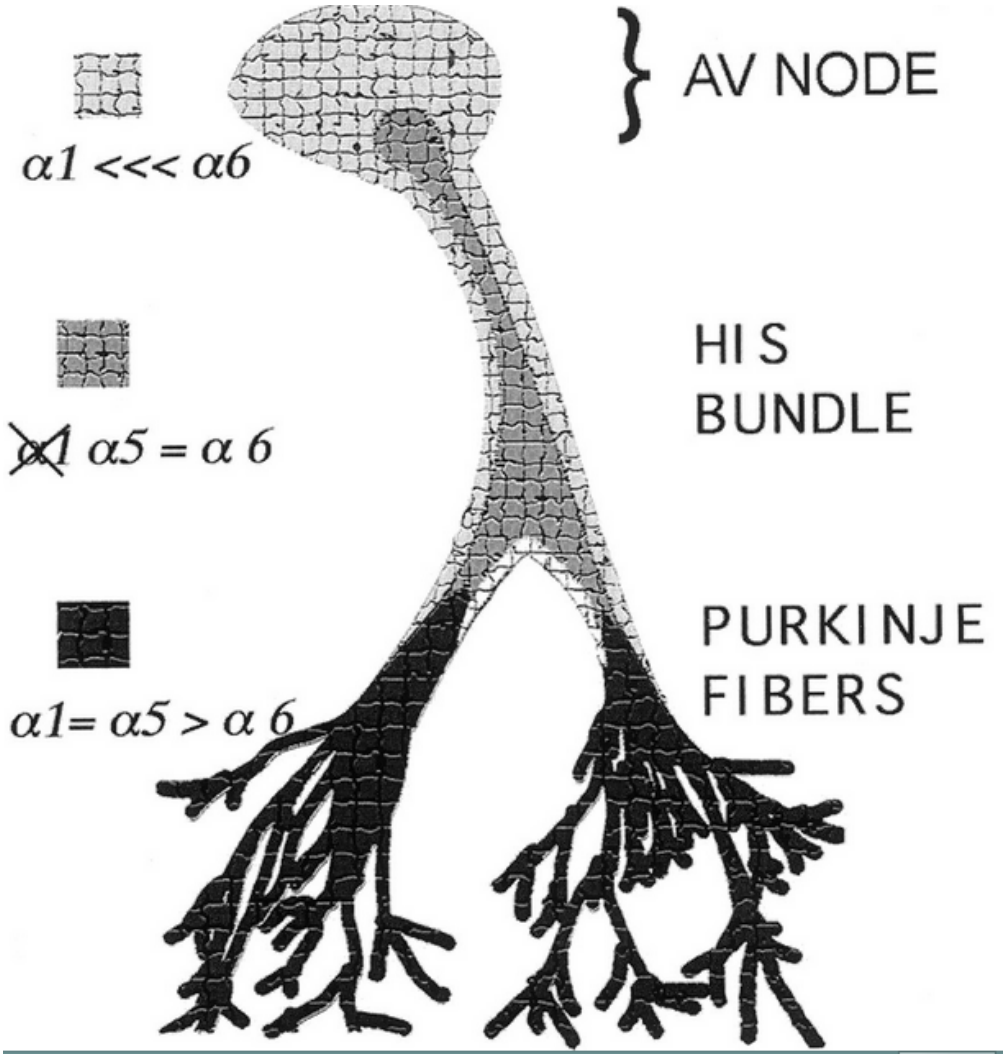
secondary effects elicited by Cx43/Cx40 deficiency in the fetal myocardium or conduction system. Given that strain background effects had previously been implicated in modifying the cardiac phenotype of the Cx40 knockout mouse,[3] is it possible that this may underlie the increased severity of heart septation defects in offspring obtained from intercrossing the Cx40 and Cx43 knockout mice? Ideally, this question can be resolved unambiguously by repeating the same experiment using Cx43 and Cx40 knockout mice maintained in an identical inbred strain background. However, in reality this often is not easy to orchestrate. Short of this, some insights may come from examining $Cx40^{-/-}/Cx43^{+/+}$ littermates obtained from intercrosses of the Cx43/Cx40 double-knockout mice and confirming that these mice exhibit the same mild heart-septation defects found in Cx40-deficient mice derived from the single Cx40 knockout mouse matings. Yet another experimental strategy is to interbreed $Cx40^{+/-}/Cx43^{+/-}$ knockout mice (representing F1 animals) generated from the Cx40/Cx43 double-knockout intercrosses and examine the resulting F2 progeny for cardiac defects. These F2 animals should be more similar to each other in strain background than the F1 animals obtained in the initial Cx43/Cx40 knockout intercross. Resolution of this strain background issue is of tantamount importance, because resting on this is our understanding of the essential roles of connexins in cardiac development and function.

The tripartite circle is now complete with the most recent production of a Cx45 knockout mouse model.[24] These mice die from heart failure at embryonic day 10, exhibiting a cardiac phenotype more complex than expected. In the absence of Cx45, heart contractions were initiated. However, there was conduction block through the atrioventricular canal, and contractions in the outflow tract were not coordinated with those of the ventricle. These results are consistent

with the previous studies showing that Cx45 is uniquely expressed in the atrioventricular canal and outflow tract.[13] [25] In addition, the Cx45 knockout embryos exhibited an endocardial cushion defect, which is hypothesized to arise from a failure of cells in the endocardium to undergo the requisite epithelial-mesenchymal transformation. These studies show that Cx45 has an indispensable role in cardiac conduction during very early stages of heart development. In addition, Cx45 has a previously unrecognized role in development of the endocardial cushion. Because cardiac contractions are initiated in the absence of Cx45, these results suggest that there is yet another connexin expressed in the early heart tube.

With the availability of knockout mouse models encompassing all 3 major connexins expressed in the heart, it will not be long before we learn what is the cardiac phenotype of the double- and triple-compound knockout animals. If we are to unravel the unique functions of the different connexins in heart conduction and development, we must take into account issues relating to the modification of cardiac phenotype by strain background effects. Given that these 3 connexins are expressed in many other tissues besides the heart, it is likely that altering the dosage of these 3 connexin genes simultaneously may result in early embryonic lethality, a problem that perhaps can be addressed using the Cre/loxP method for generating conditional knockout animals. A potential problem with Cre recombination is that, invariably, mosaicisms arise because of the <100% efficiency of Cre recombination. This will no doubt complicate the interpretation of conditional knockout experiments, especially those involving connexins, because connexins function in a noncell autonomous manner.

Overall, these knockout mouse models have confirmed that gap junctions have important roles in cardiac conduction and heart morphogenesis. As with many other genes, the phenotypes of the connexin knockout animals have not made transparent their roles in cardiac development and function. This problem is exacerbated by the fact that most cells express multiple connexins. A central question that remains is the role of Cx43 in heart conduction. This question now can be addressed using optical mapping methods to monitor surface conduction velocities in the embryonic and fetal Cx43 knockout mouse hearts (D. Vaidya, G. Morley, and J. Jalife, personal communication, August 2000). Another outstanding question is the role of Cx45 in the central conduction system, which forms relatively late in fetal development, after the time when the Cx45 knockout mouse expires. Perhaps this developmental block can be bypassed using the Cre/loxP method of conditional gene knockout. The major challenge in the future is to relate the cardiac phenotypes of the connexin knockout animals to events occurring at the cellular and molecular levels. Only then will we begin to unravel the multiple roles of gap junctions in cardiac conduction and heart morphogenesis. The beginning of the end is here, and yet it seems like we have hardly begun.



Cx43 (α1 connexin) and other connexins in the atrioventricular conduction system of the adult mouse heart. Although Cx43 is absent or coexpressed with other connexins Cx40 (α5) and Cx45 (α6), in the atria and different parts of the conduction system, including the atrioventricular node, His bundle, and Purkinje fibers, Cx43 is the only connexin expressed at significant levels by the working ventricular myocardium. Note that cellular domains of connexin expression along the atrioventricular conduction axis are depicted as distinct segment-like compartments. Reprinted with permission from Gourdie and Lo.6

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