Swapping Connexin Genes
How Big Is the Gap?
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Manipulation of the expression of specific genes in transgenic or knockout (KO) mice has become an important strategy to study the biological and physiological roles of individual cardiac proteins. Several laboratories using transgenic and knockout methodologies have made substantial progress in generating mouse models with reduced connexin expression in the heart. This has provided the unique opportunity to characterize both qualitatively and quantitatively the role of cardiac gap junction channels in electrical wave propagation and has opened many interesting questions regarding the electrophysiological consequences and proarrhythmic effects of reduced intercellular coupling in the atria, ventricles, and His-Purkinje system of neonatal and/or adult mice lacking a given connexin.

The current count of 19 connexins in the mouse genome and 20 connexins in the human genome implies a highly intriguing diversity of connexin function. But the exact nature of that diversity is not clear. Connexins can no longer be looked at as passive pores that allow ions to pass from one cell to the next. At the least, these channels are dynamic pores that are regulated by a distinct pattern of cellular states and protein interactions. It is likely that these channels are more than that. Connexins are in many ways similar to scaffolding proteins that can bind cytoskeletal proteins, protein kinases, and cellular scaffolds. Recent evidence has even suggested that some of the biological properties of connexin channels are distinct from their pore ability to form gap junctions and pass electrical information from one cell to the next

Based on the complex regulation and interactions of connexins, it would be expected that each connexin may have a distinct role and that they may not be interchangeable. That some connexins have unique functions was demonstrated by the study of Plum et al, who generated two different knock-in (KI) mouse lines in which the coding region of the connexin43 (Cx43) gene was replaced either by the coding region of Cx32 or of Cx40. In Cx43KI32 or Cx43KI40 mice, it was possible to rescue the postnatal lethality of Cx43-deficient mice; however, there were significant functional and morphological differences between the wild-type and two knock-in mouse lines, supporting the need for diversity and specialization of connexins in various cell types.

In the study by Alcoléa et al, published in this issue of Circulation Research, Cx40 is replaced with Cx45. Both of these connexins are expressed in the cardiac conduction system and might be expected to have some functional equivalence. Mice were generated with the coding sequence of Cx45 replacing that of Cx40 (Cx40KO-Cx45KI). The mice were analyzed for proper expression of the KI gene and then examined for alterations in the conduction properties of the hearts. Interestingly the Cx40KO-Cx45KI mice had several distinct patterns. The initial finding was an alteration in the surface ECG. The changes were an increased duration of the P wave, implying a slowing of atrial activation and a prolongation of the QRS complex, reflecting delayed activation of the ventricles. These studies were followed by epicardial mapping, which indicated that the ventricles had an altered pattern of activation due to an increased conduction time to the right ventricle. Analysis of conduction velocity in the atria indicated that although conduction was unchanged in the right atrium, it was significantly slowed in the left. Finally, the Cx40KO-Cx45KI mice had a significant reduction of conduction velocity in the right bundle branch. These differences suggest that Cx40 and Cx45 are not functionally equivalent.

The lack of equivalence between two connexin channels is not surprising based on the distinct characteristics of these channels. Cx40 channels have more than four times the unitary conductance of Cx45 channels and a similar number of Cx40 channels could, in theory, pass much more current than Cx45 channels. The question is then whether that difference is significant enough to account for the slowing of conduction velocity. The answer is problematic. The results of Gutstein et al suggest that a reduction of Cx43 content in the ventricle equal to 90% only results in a 50% reduction in conduction velocity. Can the reduced ion flow through Cx45 channels account for the 23% reduction in conduction velocity in the left atria? Since there is no reduction in Cx43 in these mice, it would be assumed that the Cx43 channels in the left atria would still be capable of conduction, further minimizing the effect of reduced conduction through Cx45 channels. Might differences, other than ion transport, between Cx45 and Cx43 play some role in the slowing of impulse conduction?

Alternatively, it is not unreasonable to conclude that there is a lack of any effect of Cx45 in the atria. Verheule et al described a 30% decrease in atrial conduction in Cx40 mice. This figure was derived from the right atrium, and data were not presented from the left atria. Alcoléa et al did not measure conduction velocity from the Cx40 mice directly.
but relied on the values previously recorded. It is also intriguing that the conduction velocity for Cx40−/− animals noted in the study of Alcoléa et al is substantially lower than that measured by Verheule and colleagues. As the value for atrial conduction velocity from control animals is substantially different from the previous studies and there is no value determined from Cx40−/− animals, it is not possible to accurately assess the effect of Cx45 substitution. It is possible that Cx45 is incapable of replacing Cx40 to maintain conduction in the atria.

Similar questions might be asked about the role of two distinct connexins in the specialized conduction system of the ventricles. The results presented by Alcoléa et al15 and by a previous study from the same group19 demonstrate that Cx45 can act as the primary connexin in the His bundle and left bundle branch (LBB). In contrast, in Cx40KO mice, there is a right bundle branch (RBB) block, which apparently is only partially restored by Cx45 expression. In the Cx40KO-Cx45KI mice, there is a 46% reduction of conduction velocity compared with wild-type. Again, is this simply due to the lower single-channel conductance of Cx45 or some other mechanism?

At this juncture, it is important to stress the fact that the effect of Cx40 replacement by Cx45 on intraventricular conduction is not free of controversy. To begin with, while Cx40−/− mice have normal heart rates and no arrhythmias, they have prolonged PR and QRS intervals. In the study of Verhuele et al,1 the mean value of the QRS in the wild-type mouse was 11.0 versus 16.3 ms in the Cx40−/− mouse. Similarly, Tamaddon et al20 reported a mean QRS of 14.5 ms for the wild-type and 16.8 ms for the KO mouse. Using high-resolution optical mapping, Tamaddon et al20 demonstrated that the prolonged atrioventricular and intraventricular conduction times in the Cx40−/− mouse were attributable in large measure to a reduction in the velocity of impulses propagating through the RBB. Interestingly, unlike Van Rijen et al,19 these authors did not observe complete RBB block in any of their experiments. The conduction velocity measured in Cx40−/− mice (43.7 cm/s) was reduced from that recorded from the Cx40−/− mice (74.5 cm/s). This 42% reduction is similar to the alteration in conduction velocity in the Cx40KO-Cx45KI mice relative to control. As such, the ability of Cx45 to have any effect in replacing Cx40 must be questioned. Moreover, the conduction velocity value presented by Alcoléa is substantially lower in the control animals than that reported by Tamaddon et al.20 This raises a critical issue. In the Cx40KO-Cx45KI mice, the amount of Cx45 in the RBB is substantial, representing the endogenous expression of Cx45 plus the additional expression of Cx45 under the influence of the Cx40 promoter. Under these conditions, it is difficult to explain the blocking or even slowing of conduction. Computer models of impulse propagation in model cables indicate that there would need to be a substantial reduction in coupling to result in a lowering of conduction rate. It is not clear that the different unitary conductance of Cx45 and Cx40 would explain the present results. This interpretation of the results implies a much greater functional difference between Cx40 and Cx45 than just a difference in ion transfer.

An intriguing result in the study by Alcoléa et al,15 using the Cx40KO-Cx45KI, as well as in previous studies in the Cx40−/− mouse, is the demonstration that the lack of Cx40 in the two types of mice affects propagation through the RBB much more than the LBB. In the study of Tamaddon et al,20 the absence of Cx40 in the RBB resulted in significant conduction slowing, which led downstream to delayed activation of the right ventricular free wall. While these authors were unable to optically map the LBB, they did notice that activation of the right ventricular septum occurred before the complete activation of the RBB in the Cx40−/− mice. Simultaneous optical mapping and ECG recording indicated that the activation of the RBB in those mice was delayed with respect to the start of the QRS complex. Using the Q time and right ventricular septal activation as substitute markers of LBB activation, it was concluded that the RBB was somehow more affected than the LBB. Alcoléa et al have shed substantial light on the question of whether electrical and/or structural differences between the two major His bundle branches may explain the apparently selective consequences of the absence of Cx40 on RBB conduction. First, they carried out electrode mapping experiments in both bundle branches and demonstrated that conduction velocity was significantly reduced in the RBB but not in the LBB of the Cx40KO-Cx45KI mice with respect to the wild-type and heterozygote mice. Second, while not presented in the present study, they report having replaced one allele of the Cx40 gene by enhanced green fluorescent protein (eGFP) (Cx40−/−;eGFP) and observed a significant difference in the anatomical complexity of the LBB with respect to the RBB, with the former emerging from the His bundle as a large group of separate parallel strands forming a layer on the left side of the ventricular septum, and the latter being constituted by a single thin strand. Based on the studies of Kucera et al,21 who have proposed a “pull-and-push” mechanism of slow, but nevertheless, robust conduction in branching structures, it seems reasonable to speculate that, if Cx45 was upregulated similarly in both bundle branches, the safety factor for propagation was higher in the greatly branched LBB than in the unbranched RBB. However, improved technology and further studies will be necessary to provide a definite explanation to this interesting result.

References


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