Connexin40, Bundle-Branch Block, and Propagation at the Purkinje-Myocyte Junction

Jeffrey E. Saffitz, Richard B. Schuessler

Genetic engineering in mice has created unprecedented opportunities to define the contributions of specific proteins in complex cardiological processes, but these opportunities also present significant challenges in characterizing murine cardiac function and relating insights gained into the human heart. These challenges are especially keen in defining electrophysiological phenotypes. Elucidation of the role of specific gene products in impulse propagation or arrhythmogenesis in mice requires advanced technology, particularly if the gene of interest is expressed in a restricted pattern or distribution. Such is the case in the study by Tamaddon et al appearing in this issue of Circulation Research.

This study is focused on connexin40 (Cx40), a protein that forms gap junction channels responsible for intercellular current flux. Cx40 expression in the adult heart is restricted to atrial myocytes, coronary vascular endothelium, and the His-Purkinje system. In addition to Cx40, cardiac myocytes express 2 other connexins, Cx43 and Cx45, in different amounts and combinations. A major goal of present research is to understand the biological roles of individual connexins.

Previously, Simon et al from Harvard and Kirchhoff et al from the University of Bonn independently produced Cx40 knockout mice and characterized phenotypes by electrocardiography. As predicted by the pattern of Cx40 distribution, Cx40−/− mice show prolongation of the PR interval and evidence of bundle branch block. However, going the next step to define mechanisms responsible for these alterations in the surface ECG requires a more sophisticated approach, and this is the basis for the study by Tamaddon et al. These investigators, led by Dr José Jalife, have pioneered the development and application of optical mapping to characterize electrical activation and arrhythmogenesis. Now, in a technical tour de force, optical mapping has been used to delineate patterns of ventricular activation in Cx40−/− hearts and directly visualize and characterize impulse propagation in the right bundle branch of the murine His-Purkinje system.

In wild-type mice, Tamaddon et al observed that the anterior right ventricular epicardium was activated first, followed by epicardial breakthrough on the anterior left ventricle (LV), a pattern similar to that observed in larger experimental animals and humans. In contrast, earliest breakthrough occurred in the LV in Cx40+/− mice, with later activation of the right ventricle (RV). Moreover, multiple highly variable breakthrough sites were observed on both the LV and RV surfaces of Cx40−/− mice. To additionally define mechanisms underlying these abnormal patterns of ventricular activation, Tamaddon et al mapped the activation sequence of the mouse right bundle branch for the first time. They observed that absence of Cx40 reduced propagation velocity in the right bundle branch by ~40% without apparent delay in the left bundle branch or detectable slowing in ventricular propagation velocities. Thus, altered patterns of epicardial activation seemed to be directly attributable to conduction slowing in the right bundle branch.

Several aspects of this study raise intriguing questions. Although experts may disagree on the exact interpretation of the ECG findings in Cx40−/− mice, the prolonged QRS duration, notching of the R wave, and increased duration of the S wave in lead I suggest right bundle-branch block (RBBB). This condition may occur in human patients without apparent heart disease and, under these circumstances, is considered a normal variant. With increasing age, patients may progress from incomplete to complete RBBB without demonstrable alterations in the left conduction system. Therefore, the findings of RBBB without apparent abnormalities in the left bundle branch in Cx40−/− mice are reminiscent of idiopathic RBBB and raise the testable hypothesis that alterations in Cx40 expression could play a role in the pathogenesis of RBBB in patients.

RBBB is a characteristic feature of the Brugada syndrome. Although the mechanism underlying this syndrome is associated, at least in some patients, with mutations in Na+ channels, it is noteworthy that RBBB but not LBBB occurs in Brugada syndrome patients, even in those in whom bundle branch block is not present under basal conditions but can be induced by administration of lidocaine. The marked slowing of conduction in the right bundle branch without a similar change in the left bundle branch in Cx40−/− mice raises provocative questions about the determinants of impulse propagation in these 2 major divisions of the His-Purkinje system, not only in Cx40−/− mice but also in clinical syndromes characterized by RBBB but not LBBB. As far as we know, Cx40 is expressed in both left and right bundle branches, and it seems unlikely that functional differences observed in Cx40−/− mice are attributable to disparate patterns of connexin expression in the His-Purkinje system. Tamaddon et al suggest that because the right bundle branch is anatomically thinner than the left bundle branch and action potential durations are more prolonged in the right bundle branch, a lower safety factor for antegrade conduction may...
exist in the right pathway. However, it should be stressed that because visualization of the left bundle branch is technically more difficult, Tamaddon et al did not actually map conduction in the left bundle branch. Rather, they used the first deflection of the QRS complex and RV septal activation as surrogate markers of left bundle branch activation. Moreover, the observed conduction velocity in the right bundle branch of wild-type mice was considerably slower than previously reported values in larger hearts and not that much faster than the velocity in ventricular muscle. Because the technology is being pushed to its limits in terms of spatial and, especially, temporal resolution, additional studies will be required to confirm these observations. Hopefully, with additional technical refinements, it will be possible to characterize left bundle branch activation directly in Cx40−/− mice.

As mentioned previously, cardiac myocytes express multiple connexins, and the cells of the His-Purkinje system are no exception. Other connexins must contribute to impulse propagation in the His-Purkinje system of Cx40−/− mice, and, as suggested by Tamaddon et al, Cx45 may be the most likely candidate. Expression of multiple connexins creates the possibility that individual gap junction channels will be formed by more than one protein. That such hybrid channels can form has been unequivocally demonstrated, and it seems virtually certain that they do occur naturally, but this is an emerging area of investigation, and the biological implications of heterotypic or heteromeric gap junction channels are unknown. Nevertheless, it is exciting to speculate on the potential significance of hybrid gap junction channels in creating preferential communication pathways or communication boundaries in complex multicellular organs. An obvious place in the heart where this may occur is the Purkinje fiber–ventricular myocyte junction (PMJ), which has fascinated electrophysiologists for years because of the high degree of current-to-load mismatch that occurs at this site. It is still not clear how the small amount of depolarizing current (source) provided by a thin bundle of Purkinje fibers can activate a much larger mass of ventricular muscle (sink). Elegant work from the laboratories of Rohr et al has highlighted the complex relationship between electrical coupling and conduction block. These investigators have shown that under certain circumstances, diminished coupling can actually enhance safe conduction if it creates conditions that mitigate source-sink mismatch. Because ventricular myocytes express mainly Cx43 and Purkinje fibers express Cx40 and Cx45, it is possible that disparate connexin phenotypes at the PMJ may create specific types of hybrid channels with unique properties that ensure safe conduction at this critical site. Therefore, marked derangements in the composition of gap junction channels at the PMJ could account for the abnormal patterns of ventricular activation seen in Cx40−/− mice. A recent study describing cardiac phenotypes in mice with genetic deficiencies of both Cx43 and Cx40 has ushered in double-defect models that may provide important insights into this question.

Finally, connexin-deficient mice may be particularly informative models for elucidating the functional interdependence of coupling and other complex processes such as repolarization. In an elegant study by Laurita and Rosenbaum, which also appears in this issue, the concomitant effects of repolarization gradients and anatomic barriers on unidirectional block were investigated in isolated guinea pig hearts in which a laser was used to etch a 12-mm barrier containing a 1.5-mm isthmus on the epicardial surface. Unidirectional block was critically dependent on both source-sink mismatch created by the isthmus and repolarization gradients caused by delivery of a premature stimulus. Because Purkinje cells have longer repolarization times than myocytes, reduced coupling could increase repolarization gradients at the PMJ by decreasing the entrainment of repolarization. Therefore, the combination of slow conduction and potential enhancement of repolarization gradients in Cx40−/− mice may create a potential model for studying bundle branch reentry.

The mouse is far from ideal as a model of human disease, and rigorous analysis of murine electrophysiology is difficult. Nevertheless, we have entered an era of exciting experimental work made possible by the power of mouse genetics and the development of extraordinary analytical tools.

References


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