Editorial

Measuring electrophysiological changes in transgenic mouse models of cardiovascular disease

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See article by Eloff et al. [19] (pages 681–690) in this issue.

The ability to remove selected genes within the mouse genome has proven to be an invaluable tool in the study of vertebrate biology. Many genetically modified ‘knockout’ mice possess significant morphological abnormalities that have provided substantial insights into the function of individual genes. However, the severity of the phenotype is often the drawback to using knockout mice as disease models, since many human diseases are due to subtle genetic defects. This is especially true for genetic alterations that manifest as electrophysiologically disturbances in the heart, as cardiac conduction defects, substrates for arrhythmias, and susceptibility to sudden cardiac arrest may arise without any obvious morphological abnormalities.

Cardiac function requires the organized contractility of billions of myocytes. Propagation of electrical current from cell-to-cell is mediated by gap junctions, which are membrane channels that allow the transfer of ions, metabolites and other signaling molecules between neighboring cells [1–3]. In the heart, this chemical exchange permits the rapid conduction of action potentials [3–5]. The protein components of gap junctions are referred to as connexins. These proteins transverse the membrane four times and interact with connexins on adjacent cells by homophilic binding of the extracellular domains [1–3,6]. Three distinct connexin genes are known to be expressed within the adult myocardium: Connexin40 (Cx40), Cx43 and Cx45. Cx40 is preferentially exhibited in atrial tissue and the His-Purkinje system [7,8]. The distribution of Cx45 is restricted to the atrioventricular conduction system in a pattern that is similar, although not identical, to that of Cx40 [9]. The predominant connexin of the mammalian heart is Cx43, which is expressed at high levels by the working myocardium of both the atria and ventricles [3,10,11].

Knockout mice have been generated for both Cx40 and Cx43. Cx40-deficient mice have cardiac conduction abnormalities, as shown by the prolonging of several electrocardiograph (ECG) parameters. Additionally, Cx40 null mice showed increased propensity for atrial-derived arrhythmias [12,13]. Unlike the Cx40-deficient animals, the use of Cx43 knockout mice for studying the electrophysiology of the heart has been problematic. In addition to its service in the normal function of the heart, Cx43 gap junctions play a major role in embryonic development [1,2]. Thus, homozygous Cx43 null (Cx43−/−) mice develop severe abnormalities. For example, the absence of Cx43 results in retarded ossification of the clavicles, ribs, vertebrae and limbs, and a reduced ability to regenerate myeloid and lymphoid cells [14,15]. The mice also develop cardiac malformations, which include an obstruction of the right ventricular outflow tract. As a consequence, Cx43−/− mice die postnatally of asphyxiation [16,17].

An alternative transgenic model for examining Cx43 function in the heart are heterozygous animals that possess both wild-type and mutant null alleles. As expected, mice heterozygous for the Cx43 deletion (Cx43+/−) exhibit a 50% decrease in the expression of Cx43 protein in comparison to wild-type (Cx43+/+) mice [18,19]. Moreover, there does not appear to be any compensation for this Cx43 reduction in these mice, by either elevating the expression levels of other connexin isofoms or increasing sodium channel activity [20]. Cx43+/− mice develop normally and live as long as their wild-type littermates. Defects in these mice are relatively mild, although gene
dosage does impair blood cell regeneration [15]. With regards to the heart, it was anticipated that a significant decrease in Cx43 expression would perturb the propagation of electrical current in the heart. Yet, previous results with mice heterozygous for the Cx43 deletion were equivocal, with some studies showing conduction slowing, and others finding no significant electrophysiological abnormalities in these mutant hearts [18,21,22]. The report by Elof et al. [19] in this issue of Cardiovascular Research attempts to resolve this issue by examining the electrophysiology of Cx43+/− hearts, using high resolution optical mapping methods [23].

The experimental protocol employed by Elof et al. [19] involves the ex vivo measurement of cardiac electrophysiological function. To assess the electrophysiological consequences of reduced Cx43 expression, hearts from both wild-type and Cx43+/− heterozygous mice were excised and then perfused as Langendorff preparations. For optical mapping, the hearts were continuously perfused with the voltage sensitive dye di-4-ANEPPS. Action potentials were recorded following fluorescent excitation, with the optical throughput maximized by a pair of single-lens-reflex photographic lenses, which was magnified onto a 16×16-element photodiode array. As a comparison, ECGs were also measured in parallel heart preparations from a pair of electrodes inserted into the left atrium and ventricle. The data from the optical mapping studies demonstrated in a convincing and statistically sound fashion that the conduction velocities in the heterozygous Cx43+/− hearts were approximately 30% slower than wild-type hearts. This conduction slowing was without any changes in either the pattern of conduction or anisotropic ratio, indicating that Cx43 expression was reduced uniformly throughout the myocardium of the heterozygous mutants. In contrast, ECG recordings were not able to measure any discernable differences between Cx43+/− and Cx43+/+ hearts.

Why the discrepancies with previous studies? The authors of this study considered the genetic background, since previously reported data used Cx43+/− animals of mixed genetic backgrounds. However, their results with mutant mice of mixed and congenic backgrounds were essentially identical. Thus, they conclude that differences in experimental methods account for the discrepancies in the results. The report by Morley et al. [22] that a reduction in Cx43 levels did not produce conduction slowing, was also based on optical mapping data. However, it is probable that technical factors in that previous study contributed to a lesser sensitivity in measuring conduction velocities. In the current study, the use of tandem-lens imaging and a photodiode array resulted in far higher sampling rates for recording optical signals. Interestingly, the earlier investigations that reported conduction slowing in the Cx43+/− mouse observed an even greater reduction in conduction velocities than was apparent in the current report [18,21]. For those experiments, linear electrode arrays were placed along the epicardial surface of the hearts, from which electrogams were recorded. However, in those prior studies the hearts were cooled to 31°C, which slowed the electrical activity of the hearts. In the present study, conduction velocities were measured in hearts maintained at 37°C, thus providing near physiological conditions for ascertaining cardiac function.

The inability to ascertain changes in the ECG of hearts that have reduced levels of Cx43 indicates that surface ECGs may not be sufficient to measure subtle conduction defects that might exist in various transgenic mouse models for cardiac diseases. Until recently, mice were not the animals of choice for analyzing cardiac electrophysiology. Obviously, the small size of the murine heart presents a challenge in itself. But also, the cardiac cycle length is extremely short, being no more than 10% that of humans [24]. However, with increased use and generation of different cardiac relevant transgenic mice, as well as the increasingly refined perturbations of molecular expression in these mice, the need to accurately measure subtle changes in the cardiac electrophysiology of these animals is vital. Two recent studies serve as examples of the greater sophistication of newly generated transgenic models of cardiac disorders. Plum et al. [25] report on their work with ‘knock-in’ mouse lines that involve the replacement of the Cx43 coding region with the corresponding sequences from the Cx40 and Cx32 genes. Gutstein et al. [26] describe the generation of mice with a cardiac myocyte restricted inactivation of Cx43, thereby preventing most of the major developmental abnormalities of the more traditional Cx43 knockout mouse that occur due to the absence of Cx43 in other cell populations. In both cases, the mice that were generated with these molecular perturbations suffered electrophysiological defects of the heart. Studies such as these point to future experimentation involving the replacement of individual genes by specific mutated forms of these molecules, solely within selected tissues or small groups of cells. Additionally, these animals may be challenged with a variety of environmental insults, such as changes in diet, chemical exposure or physical exertion, to determine whether minor genetic defects might make these mice more susceptible to conduction disturbances, arrhythmias, and sudden cardiac arrest. These types of investigations will require methods that can accurately measure subtle changes in cardiac electrophysiology.

The study by Elof et al. [19] makes an important contribution by demonstrating that high resolution optical mapping approaches are up to that task.

References