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$Na_V1.5$ in embryonic heart development

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The voltage-gated sodium (Na_V) channel is essential for cardiomyocyte function. It is responsible for generating the rising phase of the cardiac action potential. The channel consists of a pore-forming, α-subunit, whose main cardiac isoform is $Na_V1.5$, and one or two associated β-subunits. Moreover, it is part of a macromolecular complex including a diverse set of interacting proteins, some of which are implicated in regulating its localization to specialized membrane domains of the cardiomyocyte sarcolemma[1]. $Na_V1.5$ is required during mammalian development, since null mice for *Scn5a*, i.e. the gene coding for $Na_V1.5$, die around embryonic day (E)10.5, displaying severe defects in ventricular morphogenesis, while the heterozygotes show normal survival. A link between the Na_V channel and cardiac morphogenesis in humans has been put forward in the identification of several *SCN5A* mutations associated with structural heart disease, such as dilated cardiomyopathy.

In this issue of *Acta Physiologica*, Marchal *et al.*[2] address the implication of $Na_V1.5$ in structural development of the heart. The authors show that sodium current (I_{Na}) begins to be important for electrical activity in mouse heart at E10.5, coincident with death *in utero* of mice homozygous for *Scn5a*-1798insD; the equivalent C-terminal insertion of an Asp residue causes sudden death in heterozygous human carriers. However, given the affected cardiac growth that they observed already at E9.5, their data support that $Na_V1.5$ is implicated in structural development of the heart, thereby it has a key role earlier in embryogenesis unrelated to its electric activity. To provide evidence that I_{Na} is functionally relevant for cardiac conduction from E10.5 onwards, they performed optical mapping recordings and action potential measurements using isolated hearts. In addition, they carried out patch-clamp analyses on cardiomyocytes isolated from micro-dissected embryonic hearts. These approaches allowed the authors to conclusively show that lethality of (homozygous) *Scn5a*-1798insD$^{-/-}$ mouse embryos at E10.5 can be explained by earlier structural alterations, leading to small hearts, likely necrotic. The presence of structural abnormalities already at E9.5, i.e. at a time point during embryogenesis in which I_{Na} is not yet functionally relevant, led them to conclude that $Na_V1.5$ has crucial non-electrogenic (I_{Na}-independent) functions in early mammalian heart development.

The conclusions reached by Marchal and colleagues are supported by convincing experiments. The equivalent mutation to *Scn5a*-1798insD in humans is 1795insD, which causes an overlap syndrome of cardiac Na_V channel disease that includes QT interval prolongation[3]. It maps within a highly conserved acidic region – right after transmembrane domain D4 – which also harbors point mutation E1784K, associated with Long QT (LQT) syndrome type 3. Perhaps not coincidental, two mutations in Tyr-1795 causing opposing effects on channel gating have been described, namely, Y1795H, associated with Brugada syndrome, therefore causing loss-of-function, and Y1795C, also associated with LQT-3, thus causing gain-of-function of the Na_V channel[4]. Altogether, these data support that the $Na_V1.5$ C-terminal tail has a key role in controlling channel gating. In this regard, minor changes seem to lead to clear and differing effects on the channel's biophysical properties and to the consequent channelopathies. This example also provides additional evidence of a molecular connection between Brugada syndrome and LQT-3.

Intriguingly, this region of negatively charged amino acid residues encompasses a segment of the Na_V α subunit that interacts with fibroblast growth factor (FGF) homologous factors (FHFs); unlike FGF, FHFs cannot interact with FGF receptors and therefore must have growth-

unrelated roles. In particular, a member of the FHF family, FHF1B, binds to $Na_V1.5$ within this conserved acidic rich domain – specifically, on amino acids 1773-1832 in the human isoform – and modulates the channel's properties, albeit it does not affect the I_{Na}. Interestingly, however, FHF1B binding to $Na_V1.5$ mimics the effects of yet another LQT3-associated mutation, i.e. D1790G. Thus, it has been speculated that FHF1B expression levels may affect cardiac function; hence, maintaining relatively low FHF1B levels in the adult heart would be functionally important to prevent physiological effects comparable to those in LQT syndrome[5]. On the other hand, it is not known why the insertion of a single Asp produces such deleterious effects in $Na_V1.5$. It is quite possible that exploring further interactions within this region will provide answers to such challenging enigma.

Most likely, the *Scn5a*-1798insD mutation in homozygosis does not affect $Na_V1.5$ levels in the embryonic mouse myocardium, similarly as in adult heterozygotes[3]. Yet, the subcellular distribution of $Na_V1.5$ may be altered secondary to the mutation. However, cardiomyocytes lack distinct sarcolemma microdomains – such as intercalated discs (ID) – during early embryogenesis, when homozygous *Scn5a*-1798insD$^{-/-}$ embryos are still alive. Therefore, it is unknown how targeting to specific surface microdomains may be affected in the context of this mutation.

Taking into account the data now published[2], $Na_V1.5$ probably has a more primary role in formation of mature ID than what was initially anticipated. While embryonic cardiomyocytes lack distinct ID, these new observations suggest that $Na_V1.5$ interacts with proteins essential in the formation of ID structures. This idea opens up new avenues for exploration as to the crucial importance of Na_V channel-interacting partners within the connexome, i.e. the macromolecular complex regulating electrical coupling, cell adhesion, and cell excitability in adult cardiomyocytes[1]. Some of the proteins relevant for $Na_V1.5$ localization and functioning would include ankyrin-G, which links membrane proteins to the underlying actin cytoskeleton; the gap junction protein connexin 43; the desmosomal plakophilin-2 and plakoglobin, the latter, as a link with adherens junctions; and N-cadherin, as a cytoskeletal adaptor mediating cell-cell adhesion and clustering. On the other hand, given the overlapping localization between $Na_V1.5$ and various proteins at the other ID, it is indeed conceivable that these are transported together to their destination. In this regard, it is remarkable the finding that the MAGUK (membrane-associated guanylate kinase) CASK (calcium/calmodulin-dependent Ser protein kinase) interacts with $Na_V1.5$. Intriguingly, if knocked down, I_{Na} is increased, specifically because of its inhibitory

action on $Na_V1.5$ trafficking to the sarcolemmal lateral membrane[6]. Finally, preassembly and co-traffic of potassium channels with $Na_V1.5$ could also play a role, and the potential contribution of β subunits in establishing the localization of $Na_V1.5$ to these subdomains is also worth investigating.

Thus, it is critical to understand how $Na_V1.5$ trafficking and localization are regulated, since its proper localization is emerging as a determinant for driving formation of the ID region in developing cardiomyocytes, altogether becoming essential for proper cardiac development and embryonic survival. In summary, the new evidence that $Na_V1.5$ affects cardiac development before I_{Na} is functionally relevant for electrical function demonstrates its key non-electrogenic implication during embryogenesis. Investigating the mechanisms implicated will be of great interest to learn how dysfunction of $Na_V1.5$ may lead to cardiac structural abnormalities.

Conflict of interest

The authors declare no conflict of interest.

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