

## L-Type Calcium Channels Highs and New Lows

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Voltage-gated calcium channels are essential for coupling membrane depolarization to the influx of calcium in all excitable cells. The calcium that flows into excitable cells through voltage-gated calcium channels serves a dual function, generating both electrical and chemical signals. The intracellular events controlled by calcium are diverse and many. Excitable cells can select from a number of functionally distinct voltage-gated  $\text{Ca}^{2+}$  channel subunits, whose activities are precisely tuned to support specific tasks. These include excitation-contraction coupling in muscle, excitation-secretion coupling in neurons, hair cells, and endocrine cells, and regulation of gene expression.<sup>1–5</sup> Ten genes encode the main  $\text{Ca}_v\alpha_1$  subunit of the voltage-gated calcium channel complex in mammals.<sup>6</sup> Sequence comparisons among  $\text{Ca}_v\alpha_1$  genes from several genomes reveal three major families,  $\text{Ca}_v1\alpha_1$ ,  $\text{Ca}_v2\alpha_1$ , and  $\text{Ca}_v3\alpha_1$ .<sup>6</sup>

Even before the availability of selective toxins, several investigators demonstrated that multiple, functionally distinct classes of voltage-gated calcium channels are expressed in a variety of cell types including heart.<sup>8–11</sup> This division was based on the presence of two distinct classes of calcium channels that differed significantly in their voltage dependence of activation. The concept of low voltage-activated and high voltage-activated calcium channels was established, and although simple, this remains a useful and informative way for distinguishing among different classes of calcium channels.

Certain features have emerged from studies of voltage-gated calcium channels in heart and neurons that have established a set of standard criteria to define the presence of a specific  $\text{Ca}^{2+}$  channel subtype. Low voltage-activated, T-type,  $\text{Ca}^{2+}$  channels that contain  $\text{Ca}_v3\alpha_1$  subunits ( $\alpha_{1G}$ ,  $\alpha_{1H}$ ,  $\alpha_{1I}$ ) activate rapidly, deactivate slowly, exhibit pronounced voltage-dependent inactivation, and are insensitive to dihydropyridines and several other toxins that inhibit neuronal calcium channels. In studies of heart tissue, high voltage-activated channels have become synonymous with L-type  $\text{Ca}_v1\alpha_1$  ( $\alpha_{1C}$ ,  $\alpha_{1D}$ )-containing channels that activate with slower kinetics, but deactivate more rapidly than T-type. They exhibit weak voltage-dependent inactivation, but strong calcium-dependent inactivation, and are sensitive to dihydro-

pyridines.<sup>6,12</sup> In neurons, high voltage-activated  $\text{Ca}^{2+}$  channels are further subdivided into dihydropyridine-sensitive, L-type and dihydropyridine-insensitive, P/Q-, N-, and R-type that contain  $\text{Ca}_v2\alpha_1$  subunits ( $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1E}$ ).<sup>6,12,13</sup>

With low activation thresholds and pronounced voltage-dependent inactivation, T-type  $\text{Ca}^{2+}$  channels are optimized for contributing to depolarizing currents during the slow diastolic depolarization phase that supports pacemaking in the sinoatrial (SA) node.<sup>8,9,14,15</sup> The presence of  $\text{Ca}_v3\alpha_1$  genes in SA nodal tissue of heart supports this view.<sup>16</sup> L-type  $\text{Ca}^{2+}$  channels, on the other hand, until recently were implicated in later phases of the diastolic depolarization as the membrane potential depolarizes beyond about  $-30$  mV. Their reliance on stronger depolarization for activation is consistent with the view that L-type  $\text{Ca}^{2+}$  channels do not contribute to initiation of the action potential. However, recent studies of  $\text{Ca}_v1.3\alpha_1$  knockout mice, including those of Chiamvimonvat and colleagues reported in this issue of *Circulation Research*, offer convincing evidence supporting a role for L-type  $\text{Ca}^{2+}$  channels in action potential initiation in the SA node.<sup>17,18</sup> In both studies, mice lacking the L-type  $\text{Ca}_v1.3\alpha_1$  gene exhibit significant SA node dysfunction characterized by sinus bradycardia. Other investigators also report complete hearing loss, consistent with prominent expression of  $\text{Ca}_v1.3\alpha_1$  in inner hair cells of the cochlea.<sup>18,19</sup>

These findings are clearly paradoxical to classic descriptions of L-type  $\text{Ca}^{2+}$  channels as high voltage-activated. The explanation is relatively simple.  $\text{Ca}_v1.3\alpha_1$  L-type  $\text{Ca}^{2+}$  channels are not high voltage-activated. Evidence supporting this conclusion is presented in both the Striessnig and Chiamvimonvat studies by comparing properties of native currents in wild-type and  $\text{Ca}_v1.3\alpha_1$  knockout mice.<sup>17,18</sup> Others studies characterizing the functional properties of recently cloned  $\text{Ca}_v1.3\alpha_1$  subunits isolated from neurons and endocrine cells provide additional support.<sup>18,20–22</sup>

Striessnig and colleagues recorded from inner hair cells of the cochlea of  $\text{Ca}_v1.3\alpha_1^{-/-}$  mice and showed selective loss of a low-threshold activating  $\text{Ca}^{2+}$  current. From this they inferred the presence of a similar current in SA node cells to explain the observed abnormalities in pacemaking in the same mice.<sup>18</sup> Chiamvimonvat and colleagues now test this hypothesis directly by recording from the SA node and from isolated cells of wild-type and  $\text{Ca}_v1.3\alpha_1^{-/-}$  mice.<sup>17</sup> As reported in this issue of *Circulation Research*, the absence of  $\text{Ca}_v1.3\alpha_1$  is associated with a reduced rate of SA node firing, diastolic depolarization rate slowing at relatively hyperpolarized voltages ( $-40$  and  $-45$  mV), and the loss of calcium current in isolated SA node cells that activates at relatively hyperpolarized membrane potentials.<sup>17</sup> These new studies offer strong support that  $\text{Ca}_v1.3\alpha_1$  ablation, SA node dysfunc-

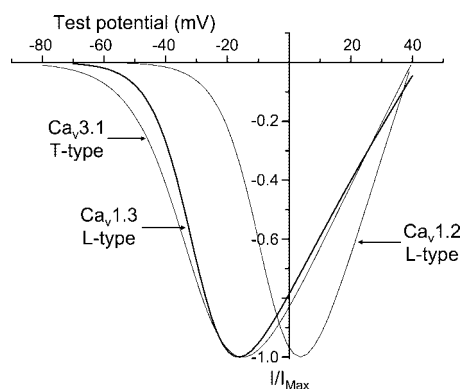
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L-type  $\text{Ca}_v1.3\alpha_1$  channels activate at negative membrane potentials similar to T-type  $\text{Ca}_v3.1\alpha_1$  channels. Normalized, peak current-voltage relationships for L-type  $\text{Ca}_v1.3\alpha_1$ , T-type  $\text{Ca}_v3.1\alpha_1$ , and L-type  $\text{Ca}_v1.2\alpha_1$  are compared. Activation mid-points ( $V_{1/2}$ ) are approximately  $-30$  mV for L-type  $\text{Ca}_v1.3\alpha_1$  and T-type  $\text{Ca}_v3.1\alpha_1$  and  $-5$  mV for L-type  $\text{Ca}_v1.2\alpha_1$ . Curves were generated by a Boltzmann-GHK function using parameters obtained from recombinant channels expressed in *Xenopus* oocytes recorded under similar conditions (10 mmol/L extracellular barium<sup>20,23</sup>).

tion, and the loss of a low-threshold activating  $\text{Ca}^{2+}$  current in SA node cells are intimately linked.

Do all L-type  $\text{Ca}^{2+}$  channels that contain  $\text{Ca}_v1.3\alpha_1$  subunit activate at hyperpolarized voltages? The answer is probably yes, based on recent functional analyses of recombinant  $\text{Ca}_v1.3\alpha_1$  channels.<sup>20–22</sup> The Figure compares peak current voltage relationships of  $\text{Ca}_v1.3\alpha_1$  L-type channels to high voltage-activated  $\text{Ca}_v1.2\alpha_1$  L-type, and to low voltage-activated  $\text{Ca}_v3.1\alpha_1$  T-type channels. The large difference in voltage dependence of activation between the two L-type  $\text{Ca}^{2+}$  channels is as striking as the similarity in the activation thresholds of  $\text{Ca}_v1.3\alpha_1$  L-type and  $\text{Ca}_v3.1\alpha_1$  T-type channels.<sup>20,23</sup> While properties of calcium channels are influenced by several factors including association with specific auxiliary subunits, the similar features of  $\text{Ca}_v1.3\alpha_1$  subunits cloned from different tissues,<sup>20–22</sup> combined with two gene ablation studies in mice,<sup>17,18</sup> favor the conclusion that low voltage-dependent activation is an intrinsic feature of  $\text{Ca}_v1.3\alpha_1$ -containing L-type  $\text{Ca}^{2+}$  channels. Clearly, significant functional differences exist among L-type  $\text{Ca}_v1\alpha_1$  genes.

If  $\text{Ca}_v1.3\alpha_1$ -containing L channels activate at hyperpolarized membrane potentials, it is rather surprising that this feature has not been highlighted in previous studies of cloned and heterologously expressed channels. Although other factors almost certainly influence channel properties, the concentration of extracellular divalent cation has large effects on the voltage dependence of activation as a result of charge screening and is a factor that differs significantly among studies. For unknown reasons, achieving high expression levels from  $\text{Ca}_v1.3\alpha_1$  clones has until recently been problematic. To compensate for low current densities, concentrations of extracellular calcium and barium up to 40 mmol/L have been used.<sup>17,24</sup> As suggested by Zhang et al,<sup>17</sup> this likely contributes to the discrepancy between properties of recombinant  $\text{Ca}_v1.3\alpha_1$  channels and the activation range expected from functional analyses of native currents in SA node cells. The use of high concentrations of extracellular divalent

cations in earlier studies of cloned channels probably obscured the unusually hyperpolarized activation range of  $\text{Ca}_v1.3\alpha_1$  L channels. It is notable that the  $\text{Ca}_v1.3\alpha_1$  L-type current-voltage relationship is shifted toward voltages  $\approx 20$  mV more depolarized and into the range of a high voltage-activated L-type channel when 40 mmol/L barium is used.<sup>20</sup>

Future studies will be needed to address the relative importance of  $\text{Ca}_v1.3\alpha_1$ -containing L-type  $\text{Ca}^{2+}$  channels in pacemaking in the heart. Although  $\text{Ca}_v1.3\alpha_1$  mRNA is present in atrial myocytes,<sup>25</sup> recent studies suggest that levels are very low in the SA node, particularly when compared with  $\text{Ca}_v3.1\alpha_1$  T-type mRNA.<sup>16</sup> The availability of a selective inhibitor of  $\text{Ca}_v1.3\alpha_1$ -containing L channels would prove a useful tool to determine the relative contribution of this channel to SA node function. Classic L-type  $\text{Ca}^{2+}$  channel blockers are not useful in this regard. Recent studies of recombinant  $\text{Ca}_v1.3\alpha_1$  L-type channels suggest a relatively low sensitivity to block by dihydropyridines compared with  $\text{Ca}_v1.2\alpha_1$  L-type channels.<sup>20,21</sup> It will be of interest to establish whether a unique splice isoform of  $\text{Ca}_v1.3\alpha_1$  is expressed in the SA node. There is evidence for some level of atrial-specific splicing of  $\text{Ca}_v1.3\alpha_1$  RNA in the S3–S4 linker of domain IV of the channel.<sup>25</sup> Splicing at this site shifts the voltage dependence of activation by  $<10$  mV and does not seem to influence dihydropyridine binding.<sup>20</sup> Finally, given the emphasis placed on similarities between  $\text{Ca}_v1.3\alpha_1$  L-type and T-type  $\text{Ca}^{2+}$  channels in terms of their activation thresholds, it is worth noting features that distinguish these channels. Whereas T-type  $\text{Ca}^{2+}$  channels undergo prominent voltage-dependent inactivation,  $\text{Ca}_v1.3\alpha_1$  L-type  $\text{Ca}^{2+}$  channels show weak voltage-dependent, but strong calcium-dependent, inactivation. Further,  $\text{Ca}_v1.3\alpha_1$  L-type  $\text{Ca}^{2+}$  channels deactivate rapidly compared with T-type  $\text{Ca}^{2+}$  channel subtypes that dominate in heart.

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