REVIEW

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Impact of gating modulation in $Ca_V1.3$ L-type calcium channels

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$Ca_V1.3$ L-type channels control inner hair cell (IHC) sensory and sinoatrial node (SAN) function, and excitability in central neurons by means of their low-voltage activation and inactivation properties. In SAN cells $Ca_V1.3$ inward calcium current (I_{Ca}) inactivates rapidly whereas in IHCs inactivation is slow. A candidate suggested in slowing $Ca_V1.3$ channel inactivation is the presynaptically located ribbon-synapse protein RIM that is expressed in immature IHCs in presynaptic compartments also expressing $Ca_V1.3$ channels. $Ca_V1.3$ channel gating is also modulated by an intramolecular C-terminal mechanism. This mechanism was elicited during analysis of human C-terminal splice variants that differ in the length of their C-terminus and that modulates the channel's negative activation range and slows calcium-dependent inactivation.

Voltage-gated L-type calcium channels (LTCCs) form the Ca_V1 channel family, which comprises the isoforms $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$ and $Ca_V1.4$. Ca_V1 channels are well-known pharmacotherapeutic targets of Ca^{2+} channel blockers such as dihydropyridines. Among the Ca_V1 family, $Ca_V1.3$ L-type channels physiologically control inner hair cell (IHC) sensory and sinoatrial node (SAN) function, as well as excitability in central neurons by means of their peculiar low-voltage activation and differential inactivation properties.[1] A lot of our knowledge about $Ca_V1.3$ channels comes from knock-out mouse models ($Ca_V1.3^{-/-}$).[1-3] $Ca_V1.3^{-/-}$ mice are deaf and suffer from sinoatrial node dysfunction.[1,2] The deafness is due to the complete absence of L-type Ca^{2+} currents (I_{Ca}) in IHCs and outer hair cells of the cochlea[1,4] where $Ca_V1.3$-mediated L-type currents comprise about 90% of the calcium current. $Ca_V1.3^{-/-}$ mice also exhibit an arrhythmic and bradycardic heart beat. This phenotype is due to an intrinsic defect in the SAN present at low heart rates.[1]

$Ca_V1.3$ channels can conduct calcium inward current in the operating range of IHCs and SAN cells (which is between -60 and -40 mV) due to their negative activation range.[1,5-7] In neurons, $Ca_V1.3$ channels shape neuronal firing as for example in striatal medium spiny neurons[8] and contribute to dendritic calcium oscillations in Dopamine (DA)-releasing neurons of the *substantia nigra* pars compacta (SNc).[9] Ca^{2+} ions entering SNc DA neurons through LTCCs elevate cellular vulnerability to toxins used to create animal models of Parkinson's disease (PD).[10] In animal models of PD, block of $Ca_V1.3$ channels appears to underlie neuroprotective therapeutic effects of dihydropyridine (DHP) LTCC blockers[10] and DHPs ameliorate the development of L-DOPA-induced dyskinesias.[11] In a retrospective human study, long-term use of calcium channel blockers was associated with a significantly reduced risk of a PD diagnosis.[12] In this context it is important to note, that DHPs act as state-dependent blockers that need the channel's inactivated state and are thereby very likely to show activity-dependent potency.

The typical low activation threshold is intrinsic to $Ca_V1.3$ channels; however, their inactivation properties vary in a wide range and seem to be differentially modulated. Whereas $Ca_V1.3$ I_{Ca} inactivates rapidly in SAN cells[1,13] its inactivation is slow in IHCs.[1] CaM-like Ca^{2+} binding proteins (CaBPs) have been shown to eliminate calcium-dependent inactivation (CDI) of a short rat $Ca_V1.3$ channel isoform by competing CaM binding to the channel's C-terminus[14,15] and also $Ca_V\beta2$ was recently reported to slightly affect CDI in IHCs,[16] but voltage-dependent inactivation remained largely unaltered. A candidate suggested in slowing $Ca_V1.3$ channel inactivation was a presynaptically located ribbon-synapse protein called Rab3-interacting molecule (RIM) that is co-localized with $Ca_V1.3$ in the same presynaptic compartments of IHCs.[17] In tsA-201 cells, RIM proteins inhibit $Ca_V1.3$ inactivation by slowing both CDI and VDI and induce a non-inactivating current component typical for $Ca_V1.3$ currents in IHCs.[17] The modulatory effects of RIM are mediated via its binding to the $Ca_V\beta$-subunit of the Ca^{2+} channel complex (**Fig. 1**). Because RIM mRNA is detected in the organ of Corti in IHC preparations before the onset of hearing, RIM proteins might therefore partly account for the slow inactivation of $Ca_V1.3$ IHC currents at least in an early developmental stage.

Furthermore, $Ca_V1.3$ channel gating is also controlled by an intramolecular protein-protein interaction in the channel's C-terminus.[18] An intrinsic C-terminal modulator (CTM) controls both activation as well as inactivation properties via binding of the distal $Ca_V1.3$ C-terminus to a more proximal domain containing the EF-hand, pre-IQ- and IQ-motif, and a regulatory domain right after the IQ-motif (**Fig. 1**) that has also been identified to be important in $Ca_V1.2$ channels.[19] Most interestingly, alternative splicing generates $Ca_V1.3$ $\alpha1$-subunits with long or short C-termini in various tissues[18] and might thereby enable tight control of channel gating. In tsA-201 cells, the absence of

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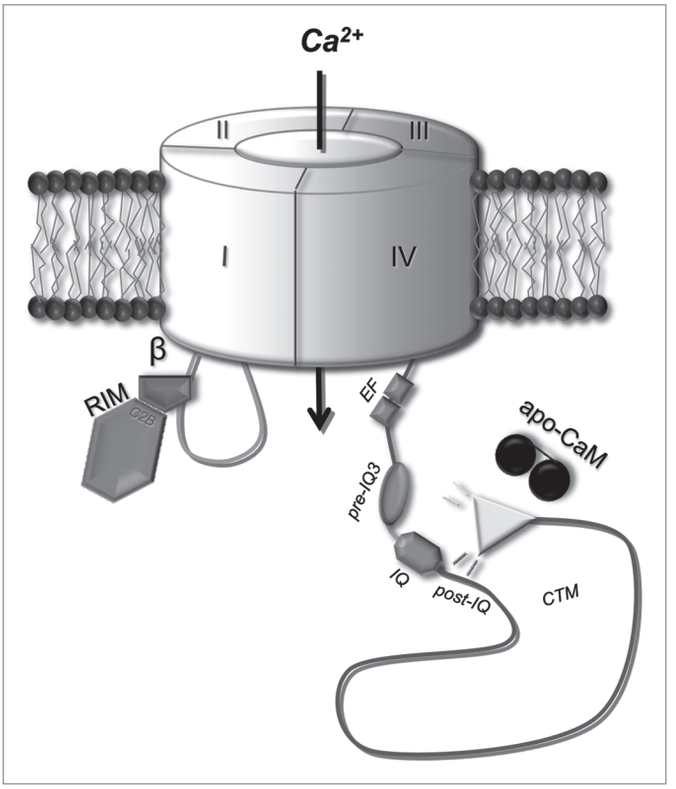

Figure 1. Cartoon of a proposed model for differential $Ca_V1.3$ gating modulation. The $Ca_V1.3$ channel is given as transmembrane pore forming α1-subunit in grey. RIM protein (pink) via its C2B domain interacts with the $Ca_V\beta$ subunit (magenta), which in turn binds the I–II loop (light blue). The C-terminus (dark blue) contains the CDI maschinery (comprising the EF hand, pre-IQ and IQ domains) and a post-IQ domain that interacts with the distal C-terminus forming the CTM in $Ca_V1.3$ channels. Modulation of the channel's CDI is suggested a competitive mechanism in which the $Ca_V1.3$ CTM competes with apoCaM for binding near the channel IQ domain.[18,23]

the CTM in a human short splice variant led to a lower activation range, a negative shift of the voltage-dependence of inactivation as well as more pronounced CDI of the channel compared to the long variant.[18] The physiological implications of the $Ca_V1.3$ CTM are still ambiguous. The CTM in long $Ca_V1.3$ channels may be suitable for longer lasting Ca^{2+} signals triggered by stronger depolarization inducing CREB phosphorylation and synaptic plasticity.[20] Differences in the CTM inactivation pattern of long and short $Ca_V1.3$ channels (e.g., due a different extend of accumulation in their inactivated state) could though underlie different shapes or firing rates of action potentials as observed in different types of neurons.[21] In terms of pharmacological intervention the $Ca_V1.3$ CTM should also have a strong impact on the efficiency of DHP block because this correlates with the amount of channels inactivated.[5,6]

Based on the emerging role of $Ca_V1.3$ for normal and potential pathological cellular function, the discovery of the $Ca_V1.3$ CTM also raises a question about its potential as an alternative concept for pharmacological modulation of $Ca_V1.3$ channels. Potential $Ca_V1.3$ selective drugs may be envisaged to have disadvantages, such as slowing of the heartbeat, as seen in $Ca_V1.3^{-/-}$ mice[1] and adaptive mechanisms seem not be able to restore pace-making in cardiac cells. The pathophysiological consequences observed in mice lacking function $Ca_V1.3$ channels about ten years ago are meanwhile also reported in two consanguineous Pakistani deafness families that also show severely impaired SAN function due to a splice variant specific $Ca_V1.3$ loss-of-function mutation.[22] Interference with only the $Ca_V1.3$ CTM interaction should be beneficial to alter cellular excitability by switching form the long to short channel gating mode in avoidance of complete channel block. Such shift in $Ca_V1.3$ gating properties within a limited range could provide also a novel strategy for therapeutic Ca^{2+} channel modulation that avoids complete state-dependent inhibition of these channels, but could nevertheless induce functional changes to obtain the desired pharmacological effects.

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