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AUTOCOMMENTARY

T-type channels: release a brake, engage a gear

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Low-voltage activated T-type calcium channels, including $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ channels, are best recognized for their negative voltage of activation and inactivation thresholds that allow them to operate near the resting membrane potential of neurons. They are typically recruited by either subthreshold membrane depolarizations, or by hyperpolarizations that remove inactivation, and are therefore perfectly suited to shape action potential threshold, and to generate a low-threshold burst discharge that occurs during physiological and pathological neuronal rhythmogenesis. In addition, they support a "window current" allowing Ca^{2+} entry at rest (for review see [1]). In contrast to the closely related high-voltage activated (HVA) channels whose gating is largely influenced by the coupling with auxiliary subunits, functional expression of T-type channels does not require the presence of any accessory subunits. Indeed, heterologous expression of the Ca_V3-subunit alone is sufficient to generate currents that mimics the currents observed in native tissue, suggesting that gating and kinetics of T-type channels are regulated by distinct mechanisms from HVA channels (for review see [1]). Structural and functional analyses have revealed the presence of a helix-loop-helix structure, so-called *gating brake*, located within the proximal 62 amino acids region of the intracellular I-II linker of the Ca_V3 subunit, and highly conserved among virtually all T-type channels including mammalians and their invertebrate orthologs.[2] Deletion of this molecular determinant gives rise to channels that activate at even more hyperpolarized potentials and present faster activation and inactivation kinetics.[3]

In our recent study,[4] we have extended the functional characterization of the *gating brake* by contrasting its importance within the Ca_V3 family members. Activation of voltage-gated calcium channels (VGCC) proceeds in 2 steps (Fig. 1). First, it requires the initial mobilization of the channel voltage-sensor (presumably formed by the S1-S4 segments), followed by the opening of the ionic pore. Activation of the voltage-sensor upon depolarization of the plasma membrane triggers the outward movement of S4 segments that produces the charge movement measurable as gating currents. Consequent opening of the pore generates the ionic current. Currently, much more information is available on the regulation of the ionic current than on the charge movement. The voltage-dependence of the pore opening (G-V) that is proportional to the number of channels opened at a given voltage is virtually identical among all 3 Ca_V3 channel isoforms. In contrast, the time constant of current activation, which reflects the transition kinetic between closed and open states of the channel, is similar between $Ca_V3.1$ and $Ca_V3.2$ channels, but of one order magnitude slower for $Ca_V3.3$ channel. Removal of the *gating brake* facilitates channel activation, manifested by the opening of the pore at lower membrane potentials, and accelerated activation kinetics. Interestingly, upon deletion of the *gating brake*, intrinsic differences between $Ca_V3.3$ and $Ca_V3.1$ channels were abolished and the $Ca_V3.3$ deletion mutant presented a similar

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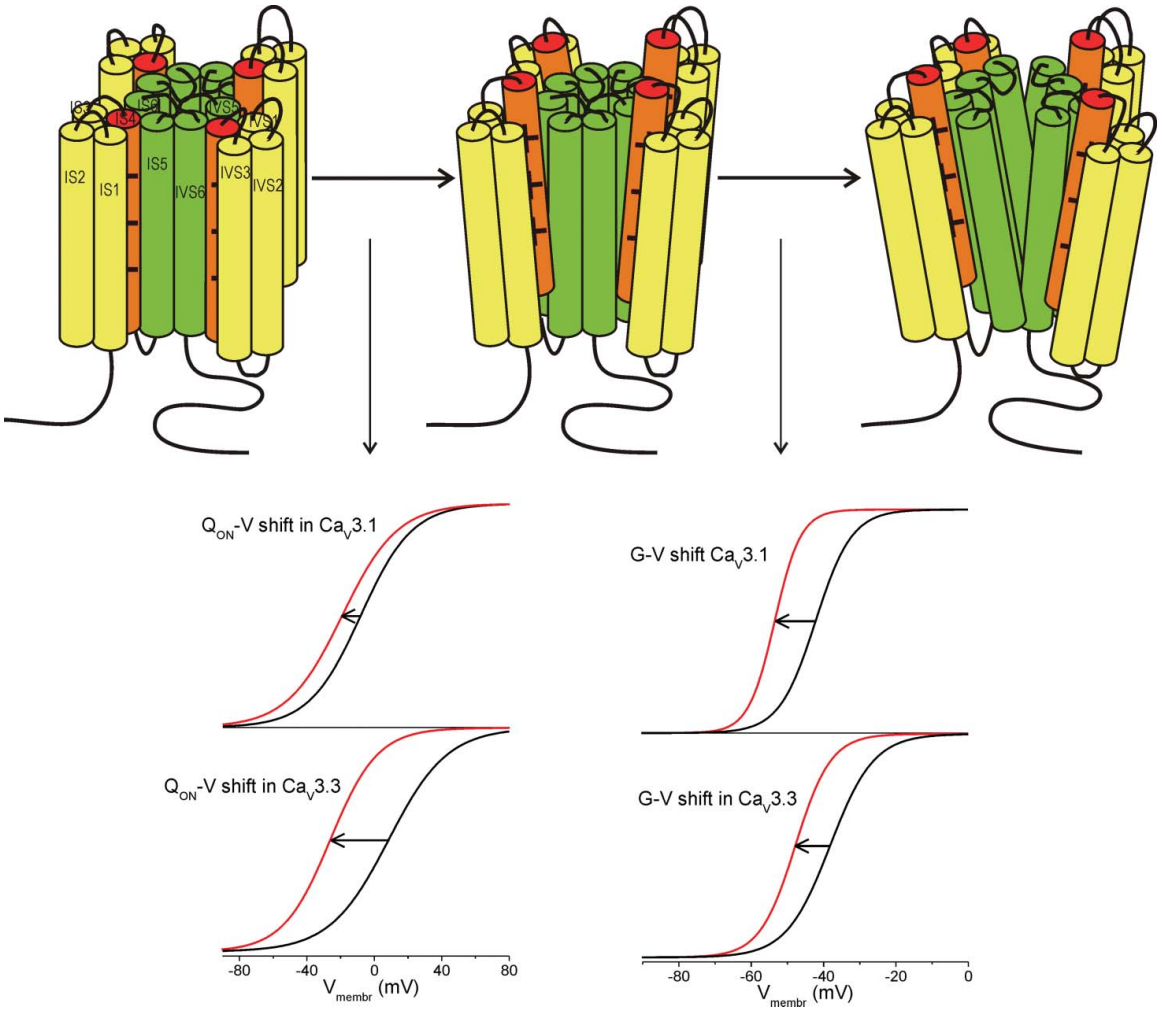

Figure 1. Schematic representation of Ca_V3 channel activation. The initial step is characterized by the mobilization of the voltage sensor (formed by S1-S4 segments, shown as yellow and orange barrels) and the upward movement of the positively charged S4 segments (orange barrels) at the origin of the ON-charge movement. Deletion of *gating brake* facilitates the movement of S4 segments which are mobilized at lower membrane depolarizations (black lines – voltage-dependences of ON-charge movement in wild type channels; red lines – voltage-dependences of ON-charge movement in channels deleted of the *gating brake*). Facilitation of the charge movement is much more prominent in the $Ca_V3.3$ channel. A second step corresponds to the pore opening (S5-S6 segments creating the channel pore are depicted as green barrels). Pore opening is manifested by the ion current. Deletion of *gating brake* shifts the voltage-dependences of the pore opening in both $Ca_V3.1$ and $Ca_V3.3$ channels to a similar extent (black lines – conductance-voltage-dependences of wild type channels; red lines – conductance-voltage-dependences of mutant channels deleted of the *gating brake*).

behavior as the $Ca_V3.1$ channel. Analysis of the charge movement revealed intriguing feature of Ca_V3 gating. In contrast to other VGCC, only a minor fraction of total charges is moved when maximal inward current is reached.[5] In addition, mobilization of the voltage-sensor occurs at significantly more positive voltages for $Ca_V3.3$ channel compared to $Ca_V3.1$ channel, as evidenced by a depolarized shift the voltage-dependence of the ON-charge (Q_{ON}-V).[4] From a thermodynamic point of view, this indicates that more energy is needed to mobilize the voltage-sensor of $Ca_V3.3$ channels than of $Ca_V3.1$ channels. On the other hand, because the voltage-dependences of pore opening are nearly identical, it implies that less charge transfer is needed to open the pore of $Ca_V3.3$ channels than of $Ca_V3.1$ channels suggesting a more efficient coupling or "gear" between the activation of the voltage-sensor and the pore opening. We and other groups previously demonstrated that removing the *gating brake* in Ca_V3 channels doubled the slope of G_{max}-Q_{ONmax} relationship,[3,6] suggesting an improved "gear." In addition, removing the gating brake in $Ca_V3.1$ and $Ca_V3.3$ channels resulted in a hyperpolarized shift of the voltage-dependence of the charge movement, indicating

that less energy is required to move the voltage-sensor and that the *gating brake* regulates not only the pore opening but also the activation of the voltage sensor. Shift caused by the deletion of the *gating brake* in $Ca_V3.3$ channels was of much greater magnitude, suggesting that the *gating brake* in $Ca_V3.3$ channels is "braking" more than in $Ca_V3.1$ channels (Fig. 1).[4] In addition, the kinetics of the ON-charge movement were found significantly accelerated upon deletion of the *gating brake* in the $Ca_V3.3$ channel, suggesting an increased mobility of the voltage-sensor.

The exact molecular mechanism by which the *gating brake* modulates both steps of T-type channel gating (voltage sensor activation and pore opening) remains elusive. Amino acids forming the *gating brake* immediately follow the pore forming IS6 segment, but is not connected to any voltage-sensor. It is possible that the *gating brake* could interact with some of the molecular determinants of the voltage-sensor, and decreasing its mobility. Molecular modeling demonstrated that the *gating brake* might interact directly with the IS4-S5 linker.[2] However, up-to-date, there is no biochemical evidence for such an interaction. In addition, the molecular mechanistic linking the voltage-sensor to the pore opening remains to be explored in more details. Other channel structural determinants have been identified to play a role in channel gating, including the relatively short intracellular III-IV linker. This domain is not only important for channel gating, but also contributes to channel surface expression via the ubiquitin pathway.[7] In addition, we previously demonstrated that binding of syntaxin-1A onto the Ca_V3-subunit significantly shifts the voltage-dependence of the pore opening toward more negative voltages, suggesting an increased "gear."[8] This possibly suggests the existence of intramolecular interactions within the Ca_V3-subunit and involving various molecular determinants essential for the proper channel gating. Further investigations will be necessary to uncover this complex structure and better understand the molecular mechanistic underlying the unique biophysical features of T-type channels.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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