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RESEARCH NEWS

$Ca_v\beta$ dances the two-step with VSD II

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JGP study (De Giorgis et al. https://doi.org/10.1085/jgp.202413739) reveals that the auxiliary $Ca_v\beta_3$ subunit regulates the cardiac calcium channel $Ca_v1.2$ by modulating the two-step activation of VSD II.

Voltage-gated calcium channels (Ca_vs) control a variety of physiological processes, from muscle contraction to neurotransmitter release. The pore-forming α_1 subunits of Ca_vs have four voltage-sensing domains (VSDs), each of which contains a positively charged transmembrane segment (S4) that moves in response to membrane depolarization, triggering pore opening and calcium influx. Channel opening is also regulated by $\alpha_2\delta$ and β subunits on the outer and inner surfaces of the plasma membrane, respectively, but how β subunits, in particular, affect VSD movements is unknown. In this issue of *JGP*, De Giorgis et al. reveal that the β_3 subunit promotes the opening of cardiac $Ca_v1.2$ channels by stabilizing VSD II in an intermediate, partially active position (1).

In 1993, Alan Neely and colleagues found that the β subunit shifts the voltage dependence of $Ca_v1.2$ opening so that they mediate Ca^{2+} influx at more negative membrane potentials (2). Surprisingly, though, the β subunit had no effect on $Ca_V1.2$ gating currents, which arise from the movement of positively charged residues in the channel's VSDs. "The fact that the β subunit promotes channel opening without affecting voltage-sensor movement couldn't be reconciled with the prevailing view that voltage-sensor movement was obligatory for channel opening," says Neely, now a professor at the University of Valparaíso in Chile. "This remained an unsolved puzzle for over 30 years!"

One possible explanation lies in the fact that Ca_v gating currents represent the sum of all four VSD movements and are influenced not only by the number of charged residues moved across the membrane but also by the speed at which they move. Voltage-clamp fluorometry (VCF), however, can trace even the slowest movements of individual VSDs: voltage-dependent changes in the fluorescence of fluorophores attached to the S4 segment reflect changes in the fluorophore's local environment as the VSD moves in response to membrane depolarization.

(Left to right) Daniela De Giorgis, Guido Mellado, Jose Antonio Garate, and Alan Neely.

Using this technique, researchers have found that VSD II and VSD III are the main drivers of $Ca_v1.2$ opening (3) and that the auxiliary subunit $\alpha_2\delta$ promotes this process by enhancing the coupling between these two VSDs and the pore domain (4). Now, Neely, along with first author Daniela De Giorgis, Guido Mellado, and co-corresponding author Jose Antonio Garate, used VCF to investigate how the β subunit regulates $Ca_v1.2$ opening and VSD movement (1).

As expected—given their limited involvement in $Ca_v1.2$ opening—the movements of VSD I and VSD IV were unaffected by the presence or absence of $Ca_v\beta_3$. More surprisingly, the β subunit also had little effect on the behavior of VSD III.

To analyze the movements of VSD II by VCF, De Giorgis et al. attached a fluorophore to its S4 segment and introduced a tryptophan to a nearby region of the $Ca_v1.2$ pore domain that could potentially quench the fluorescent signal as the VSD II undergoes voltage-induced movements. Notably, in the absence of $Ca_v\beta_3$, the researchers observed a biphasic change in voltage-dependent fluorescence: the fluorescent signal decreased upon mild depolarization but increased at more positive membrane potentials. This suggests that VSD II activates via a two-step process, passing from an inactive to an active conformation via a third, intermediate configuration in which the fluorescent signal is maximally quenched.

The biphasic behavior of VSD II was maintained in the presence of $Ca_v\beta_3$, but the intermediate phase was enhanced: the fluorescent signal became quenched at more negative membrane potentials than it did in the absence of $Ca_v\beta_3$, and greater depolarizations were required to unquench the signal.

To gain structural insights into their VCF data and the intermediate state of VSD II, De Giorgis et al. performed molecular dynamics simulations based on two recent cryo-EM structures of $Ca_v1.2$ (5, 6). In simulations of $Ca_v1.2$ in its resting state (5), the fluorophore attached to VSD II did not come close to the quenching tryptophan in the channel's pore

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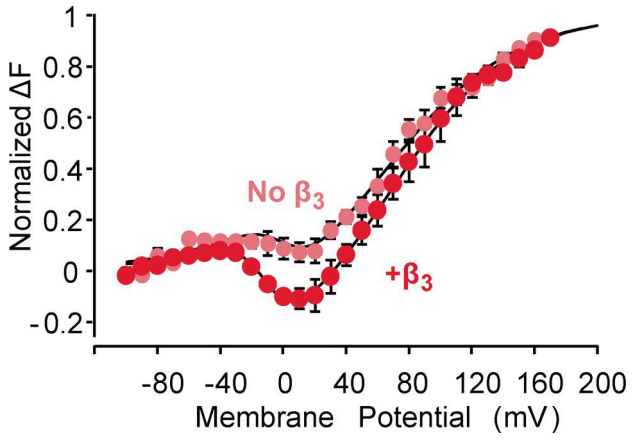

VCF reveals that $Ca_v1.2$'s VSD II displays a biphasic voltage-dependent fluorescence whose intermediate, negative phase is enhanced in the presence of the auxiliary $Ca_v\beta_3$ subunit. This suggests that $Ca_v\beta_3$ promotes $Ca_v1.2$ opening by modulating the two-step activation of VSD II.

domain. In simulations of a $Ca_v1.2$ state thought to be partially active (6), the fluorophore was positioned close enough to the pore domain tryptophan for the fluorescent signal to be quenched.

De Giorgis et al. were able to recapitulate their data using an allosteric model of $Ca_v1.2$ activation adapted to include an intermediate, partially active conformation of VSD II that is optimally coupled to channel opening. $Ca_v\beta_3$ appears to promote $Ca_v1.2$ activation by stabilizing this intermediate conformation.

Neely and colleagues now want to validate their allosteric model of $Ca_v1.2$ activation. "We are currently developing experimental strategies to demonstrate that the coupling between VSDs and the pore is allosteric, meaning that the channel can open even when all VSDs are resting, as is the case in calcium-activated potassium channels," Neely says.

References

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