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RESEARCH NEWS

Phosphoinositide regulation of voltage-gated sodium channels

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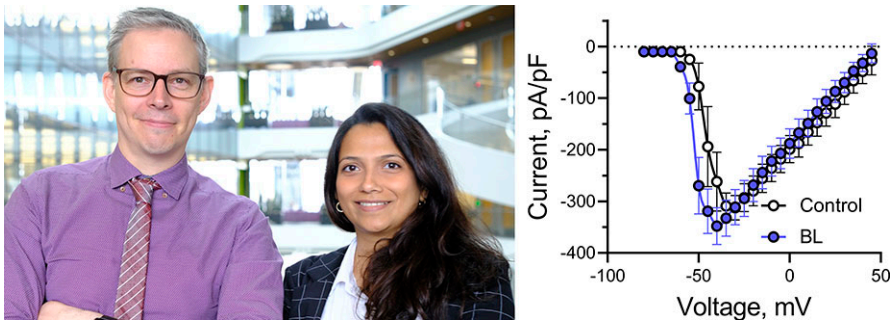
JGP study (Gada et al. 2023. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213255) reveals that the membrane lipid $PI(4,5)P_2$ alters the activity of $Na_V1.4$ channels by modulating their gating behavior.

The plasma membrane phospholipid $PI(4,5)P_2$, whose levels can be regulated by a wide variety of cell signaling pathways, is a necessary cofactor for most ion channels, modulating the gating behavior of, for example, both inwardly rectifying potassium channels and voltage-activated calcium channels (1, 2). In this issue of *JGP*, Gada et al. show, for the first time, that $PI(4,5)P_2$ also regulates the gating of a voltage-activated sodium (Na_V) channel (3).

Na_V channels are activated in response to membrane depolarization, allowing a rapid influx of Na^+ ions that initiates action potentials in a variety of excitable cell types, including neurons, cardiomyocytes, and skeletal muscle fibers (4). The channels then undergo fast inactivation, making the cells refractory to further excitation until the membrane potential returns to normal. The gating of Na_V channels is therefore tightly regulated, and defects in Na_V activity are associated with a variety of diseases, including cardiac arrhythmias, epilepsy, and skeletal muscle disorders (5). "It's notable that, among all the channels regulated by $PI(4,5)P_2$, there are no robust reports of this phospholipid regulating any Na_V channels," says Leigh Plant, an assistant professor at Northeastern University.

Plant and colleagues, including postdoc Kirin Gada, decided to investigate whether $PI(4,5)P_2$ regulates the activity of the skeletal muscle Na_V channel $Na_V1.4$ (3). "Because of its high spatiotemporal precision, we used an optogenetic technique to deplete $PI(4,5)P_2$," Gada explains. In this system, blue light triggers the recruitment of a lipid phosphatase to the plasma membrane of HEK293T cells, causing a rapid reduction in $PI(4,5)P_2$ levels.



Leigh Plant (left), Kirin Gada (right), and colleagues reveal that the phospholipid $PI(4,5)P_2$ regulates the gating of the voltage-gated sodium channel $Na_V1.4$. Compared to control conditions (black line, right), depleting plasma membrane $PI(4,5)P_2$ (blue line) shifts the voltage-dependent activation of $Na_V1.4$ by approximately −10 mV. $PI(4,5)P_2$ depletion also alters the rate and extent of fast inactivation, as well as the channel's recovery from fast inactivation. BL, blue light.

By expressing $Na_V1.4$ in these cells and subjecting them to whole cell patch-clamping, the researchers were able to compare the channel's activity before and after $PI(4,5)P_2$ depletion. Removing $PI(4,5)P_2$ left shifted the voltage-dependent activation of $Na_V1.4$ to more negative membrane potentials. It also slowed the rate of fast inactivation and increased the "late" current that persists after channel inactivation. "Moreover, recovery from fast inactivation is faster when $PI(4,5)P_2$ is depleted," Gada says.

Supplementing cells with a $PI(4,5)P_2$ analog reversed the effects of $PI(4,5)P_2$ depletion on both channel activation and fast inactivation. These two gating processes are mechanically coupled in Na_V channels, and mutations in $Na_V1.4$ that abolish fast inactivation also alter the channel's voltage-dependent activation. Notably, $PI(4,5)P_2$ depletion had no effect on the activity of these mutant $Na_V1.4$ channels, suggesting that the phosphoinositide is crucial for coupling $Na_V1.4$ activation and inactivation.

"Overall, we conclude that $PI(4,5)P_2$ is required for the channel to operate normally," Plant explains. "When we remove $PI(4,5)P_2$, we break the machine and disrupt the equilibrium between the different states of the channel."

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Plant and Gada now want to investigate whether $PI(4,5)P_2$ regulates other members of the Na_V channel family, and also whether other phosphoinositides have distinct effects on channel activity. A hint that the latter might be true comes from the fact that Gada et al. saw slightly different effects on $Na_V1.4$ gating depending upon which phosphoinositide species predominated, PI(4)P or PI, after individual $PI(4,5)P_2$ phosphatases were optogenetically activated. With the overarching goal being the development of drugs and pharmacologic strategies, it will also be important to investigate whether defects in this regulation contribute to any $Na_V1.4$-associated diseases, as well as to determine exactly how $PI(4,5)P_2$ binds and regulates $Na_V1.4$ channels.

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