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RESEARCH NEWS

Cholesterol pools cooperate to modulate HCN channels

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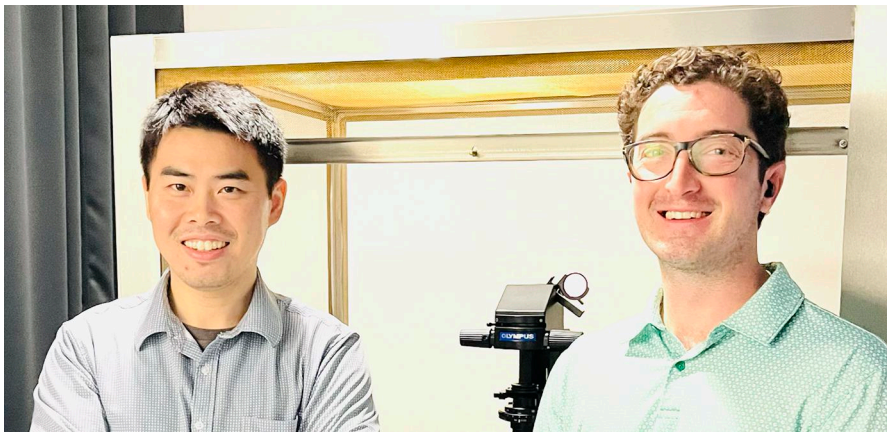
JGP study (Handlin et al. https://doi.org/10.1085/jgp.202513925) provides new mechanistic insights into the cholesterol-dependent modulation of pain sensation by DRG neurons.

Cholesterol is an abundant component of the plasma membrane, influencing the structure and function of the lipid bilayer and the proteins—including ion channels—embedded within it. In this issue of *JGP*, Handlin et al. reveal that both free cholesterol and cholesterol-dependent ordered membrane domains (OMDs) modulate the activity of hyperpolarization-activated cyclic-nucleotide gated (HCN) channels in nociceptive DRG neurons, and that the alteration of these pools after injury contributes to neuropathic pain (1).

HCN channels regulate neuronal excitability by mediating inward-directed sodium currents. Upregulation or abnormal gating of HCN channels has been implicated in the increased excitability and firing of neurons during chronic pain (2, 3).

Recently, Gucan Dai and colleagues at St. Louis University School of Medicine, found that some HCN isoforms preferentially localize to OMDs (4). The increased thickness of these domains modulates the movement of the HCN voltage-sensing S4 helix, which is twice as long as the S4 helix in other voltage-gated ion channels. In nociceptive DRG neurons, the predominant HCN isoform, HCN2, localizes to OMDs, and disruption of these domains by cholesterol extraction increases HCN channel activity and neuronal excitability (5). Notably, these OMDs are also disrupted by nerve injury in a rat model of neuropathic pain.

In addition to OMDs, however, HCN channels may also be modulated by free cholesterol in the plasma membrane: cryo-EM structures have identified a potential cholesterol-binding site near the S4 helix of HCN3 (6). "So, we wanted to investigate whether HCNs could be differentially modulated by free cholesterol and cholesterol within OMDs," Dai explains.



Gucan Dai and Lucas Handlin.

Dai and colleagues, led by first author Lucas Handlin, used specific reporters to distinguish the different pools of membrane cholesterol in DRG neurons. Free cholesterol was monitored using a fluorescent probe, eGFP-GRAM-W, that binds to accessible cholesterol in the inner leaflet of the plasma membrane. OMDs, on the other hand, were monitored using a cholera toxin subunit B–based FLIM-FRET approach, in which FRET efficiency increases as OMDs grow larger and incorporate more cholera toxin subunit B.

Using these tools, Handlin et al. found that the two pools of cholesterol show distinct responses to cholesterol supplementation (1). Naive DRG neurons, which already have large OMDs, displayed only minimal increases in OMD size after cholesterol addition, while the free cholesterol pool gradually increased over a 15 min time period. Neuropathic DRG neurons, in contrast, had small OMDs and lower free cholesterol levels to begin with. They showed a rapid increase in free cholesterol and OMD size for the first 5 min of cholesterol supplementation. After 5 min, free cholesterol levels continued to increase but OMD expansion ceased, perhaps because other OMD components, such as sphingomyelin, become limiting.

Dai and colleagues then compared the distinct dynamics of the two cholesterol pools with the effects of cholesterol supplementation

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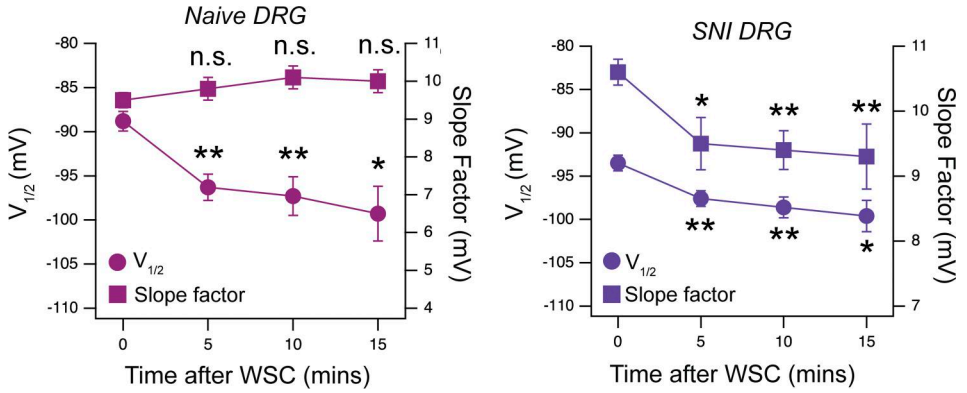

In naive DRG neurons (left), which already have large OMDs, supplementation with water-soluble cholesterol (WSC) has no effect on the slope factor, V_S, but increasingly suppresses $V_{1/2}$ as free cholesterol levels increase within the plasma membrane. In neuropathic DRG neurons (right), which have smaller OMDs and lower free cholesterol levels, cholesterol supplantation reduces both $V_{1/2}$ and V_S, particularly in the first 5 min.

on HCN gating, monitoring HCN currents in the same cells over time.

In naive DRG neurons, cholesterol addition reduced current amplitude, left-shifted the half-maximal activation voltage ($V_{1/2}$), and enhanced channel activation kinetics. These effects grew more pronounced the longer cells were supplied with extra cholesterol. The slope factor of the G-V curve (V_S) reflects the steepness of voltage dependence, from which the relative gating charge movement of voltage sensors can be inferred. V_s remained unchanged throughout the time course of cholesterol supplementation, correlating with the lack of OMD expansion. In contrast, cholesterol extraction disrupted OMDs and increased V_S.

In neuropathic DRG neurons, V_S declined dramatically in the first 5 min of cholesterol supplementation, presumably due to the rapid increase in OMD size. Taken together, these results suggest that cholesterol-dependent OMDs impact voltage-sensor movement, but other parameters of HCN gating are influenced by free cholesterol. "We think that both of these mechanisms contribute to the modulation of HCN activity," Dai says.

This modulation could represent a new therapeutic strategy for neuropathic pain. Handlin et al. found that localized cholesterol application reduced mechanical hypersensitivity in a rat pain model. The researchers now want to investigate how cholesterol levels are reduced following nerve injury, as well as confirming that free cholesterol can regulate HCN2 by directly binding to the channel.

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