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RESEARCH NEWS

Steady-state measurement of charge-voltage curves

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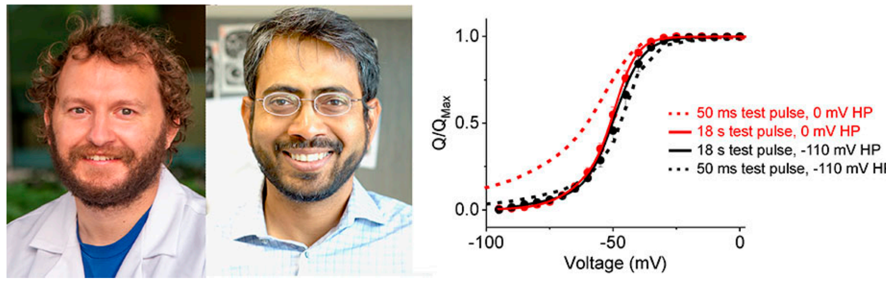
A new gating current recording protocol shows that gating hysteresis is a kinetic phenomenon, rather than an inherent thermodynamic property of Shaker potassium channels.



John Cowgill (left) and Baron Chanda (center) reveal that gating hysteresis is a kinetic phenomenon, rather than a fundamental thermodynamic property of the Shaker potassium channel. Q-V curves obtained using short test pulses of 50 ms (dotted lines) are different, depending on whether the pulses are hyperpolarizing (red) or depolarizing (black). But the curves overlap when the test pulses are extended to 18 s (solid lines) so that the gating charge movements can reach equilibrium.

Changes in membrane potential activate voltage-gated ion channels by triggering the movement of charged residues in the channel's transmembrane domains. These charge movements can be measured by recording gating currents at different membrane voltages, and the resulting charge-voltage (Q-V) curves can, in theory, be used to calculate the net free energy of channel activation.

Yet most voltage-gated ion channels display a phenomenon known as gating hysteresis, in which experimentally measured Q-V curves differ depending on the starting conditions. For the Shaker potassium channel, for example, the Q-V curve obtained by hyperpolarizing from a holding potential of 0 mV is left-shifted compared to the Q-V curve obtained by depolarizing from a holding potential of −80 mV (1). This implies that the net free energy of Shaker activation is larger than the net free energy of Shaker deactivation, and some researchers have suggested that this is an inherent thermodynamic property of the channel that arises from a process of energy dissipation during channel activation (2).

Well-established kinetic models of Shaker gating, however, do not include any loss or gain of energy during the gating cycle (3). These models also indicate that there should be no gating hysteresis at steady state and thus, explains Baron Chanda from the Washington University School of Medicine in St. Louis, the experimentally observed hysteresis may simply be a consequence of current protocols that, because they are limited to brief test pulses of <500 ms, likely measure gating currents and Q-V curves under non-equilibrium conditions. "We therefore needed to find a way to make these measurements under steady-state conditions," Chanda says.

As described in this issue of *JGP* (4), Chanda, along with graduate student, John Cowgill, devised a modified "return to reference" protocol that enables gating currents to be recorded after ultra-long test pulses that provide enough time for charge movements to reach equilibrium. Cowgill and Chanda found that gating hysteresis was eliminated with test pulses 18 s in length: the Q-V curve obtained using hyperpolarizing pulses overlaps with the Q-V curve obtained using depolarizing pulses.

The researchers complemented their findings by using voltage-clamp fluorometry to track conformational changes in the Shaker channel's voltage-sensing domain. This approach confirmed that movements within the voltage sensor are slow at intermediate potentials, and take >10 s to reach equilibrium. Accordingly, at early, non-equilibrated timepoints, fluorescence-voltage curves obtained using hyperpolarizing and depolarizing pulses are hysteretic, but the curves overlap at later, equilibrated timepoints.

"Taken together, our results show that gating hysteresis is a kinetic phenomenon rather than a thermodynamic issue," says Chanda, who notes that, because channels are usually not at equilibrium in vivo, hysteretic gating behavior can still have important physiological implications.

More importantly, in addition to explaining the origins of gating hysteresis, the protocols developed by Cowgill and Chanda enable rigorous estimation of the free energy of voltage-gated ion channel activation by identifying conditions that eliminate hysteresis. By definition, free energy is a state function and, hence, completely path independent. Thus, these new gating current recording protocols can be used to carry out thermodynamic analyses of channel gating and obtain rigorous quantitative estimates of the impact of individual mutations.

References

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