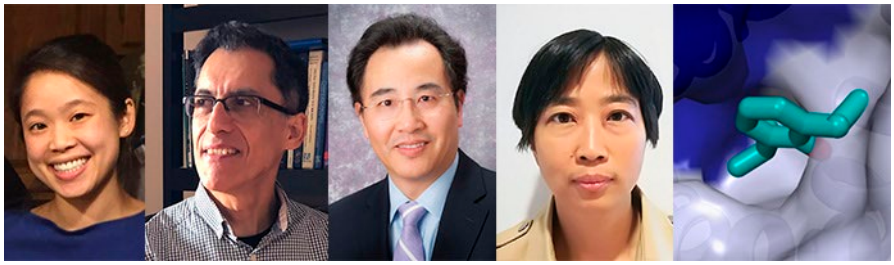
JGP Journal of General Physiology

RESEARCH NEWS

Propofol's paradox, explained

Caitlin Sedwick

Tandem *JGP* studies investigate how propofol affects voltage-gated sodium channels.



Elaine Yang and Manuel Covarrubias, in parallel with Yali Wang and Yan Xu, authored tandem *JGP* papers investigating how propofol achieves inhibition of voltage-gated sodium channels. Both labs collaborated with colleagues in a multi-institutional investigatory group (not shown) studying the mechanisms of anesthesia. At right: Molecular dynamics simulation of propofol interacting with the channel. Photos courtesy of the authors.

Almost 170 years ago, the first use of general anesthetics revolutionized medical practice by allowing doctors to block pain and induce unconsciousness in patients undergoing major surgeries. Despite their ubiquity in medical settings and intensive study in experimental ones, it's still not clear how anesthetics achieve their effects. For example, the injected anesthetic propofol was long thought to work by altering the activity of ligand-gated ion channels, such as $GABA_A$ receptors, in the brain. However, propofol has also been shown to affect voltage-gated ion channels, including the sodium channels essential for generation of action potentials in all excitable cell types (1). Two papers appearing this month in *JGP* explore the molecular mechanisms and biophysical consequences of propofol binding to voltage-gated sodium channels (2, 3).

"One of the reasons why the problem of general anesthesia is so complex is because it involves many interactions. These compounds are very nonselective," explains Dr. Manuel Covarrubias, from the Department of Neuroscience at Thomas Jefferson University in Philadelphia.

"We believe that the sodium channel should be considered one of the targets for general anesthetics. That's why we're looking very closely into sodium channels," elaborates Dr. Yan Xu, from the Department of Anesthesiology at the University of Pittsburgh.

Both Covarrubias's laboratory and Xu's laboratory have been studying how anesthetics interact with ion channels for decades. Both groups are also participants in a multi-institutional, multidisciplinary effort dedicated to untangling the mysteries of general anesthesia. Prior studies have shown that local anesthetics prevent the passage of ions through voltage-gated sodium channels by clogging the channel pore and stabilizing the channel in its inactivated state (4), which cannot conduct sodium ions. However, whether general anesthetics act through similar mechanisms is still being debated (5, 6).

For insights about how propofol affects sodium channel activity, Covarrubias's group, with graduate student and lead author Elaine Yang, used patch clamp techniques to study the biophysical properties of two bacterial sodium channels (NaChBac and NavMs) in the presence of clinically relevant propofol concentrations. They observed that propofol caused these channels to inactivate more quickly and at more negative transmembrane voltages than normal.

"If we would have stopped there, we would have said, 'Oh yeah, it looks like it's just very reminiscent of local anesthetic action,'" says Covarrubias. But Yang et al. continued their investigation by examining how a NaChBac mutant that cannot inactivate is affected by propofol. The scientists predicted that if propofol stabilizes the channel's inactive state, it should have no effect on this mutant. Conversely, if it blocks the channel pore, then it should inhibit current through the mutant. However, to their surprise, they found neither of these predictions was borne out. Instead, in an apparent paradox, propofol actually made the mutant channel more likely to open.

Additional experiments indicated that propofol does not affect the time the channel takes to recover from inactivation, confirming that the anesthetic does not stabilize the channel's inactivated state. Furthermore, propofol affected NaChBac and NavMs similarly. Yang et al. explain that propofol's inhibitory action likely arises from a well-known property of voltage-gated ion channels: that once opened, these channels inactivate. Therefore, by facilitating channel opening, propofol also accelerates channel inactivation.

How does propofol cause this change? This was the question Dr. Xu's group sought to answer by determining where the anesthetic molecule binds to sodium channels. Postdoc Yali Wang started by enlisting Dr. Pei Tang's laboratory at the University of Pittsburgh and Dr. Vincenzo Carnevale's laboratory at Temple University in Philadelphia to conduct computational docking and molecular dynamics simulations to predict likely binding sites for propofol on NaChBac. The simulations highlighted multiple potential binding sites, so Wang et al. evaluated whether binding actually takes place

csedwick@gmail.com.

at each site using saturation transfer difference nuclear magnetic resonance (STD-NMR) spectroscopy. This required inserting a small fluorine probe into NaChBac near a predicted site of interaction, then using NMR to detect transfer of nuclear spin alignment between that site and bound, fluoridated propofol (4-fluoropropofol).

Elaine Yang helped Xu's group verify that 4-fluoropropofol affects NaChBac in the same way as propofol, and that the mutations needed to insert fluorine probes into NaChBac did not affect channel function. Then, Wang conducted the NMR tests.

"We identified three important regions in voltage-gated sodium channels that we believe mediate different aspects of propofol inhibition," notes Xu. Propofol binding was observed at the channel's voltage-sensing domain (VSD); within the channel pore, near the selectivity filter; and on the intracellular face of the channel, at a region linking the transmembrane helices S4 and S5.

Interestingly, Covarrubias's group, who also collaborated with Carnevale's laboratory to predict propofol binding sites in NaChBac and NavMs, likewise identified the S4-S5 linker region as potentially important. They propose that propofol binding at this site could spur channel activation. But as Xu points out, all three interacting regions are likely involved in mediating propofol's effects. For example, propofol binding at the VSD may help promote channel activation, and binding near the selectivity filter could promote inactivation and block the channel pore. Electrophysiological data indicate such blocking can occur, but only when propofol is present at concentrations higher than those Covarrubias's group tested.

"We still need more studies to pinpoint the contribution of each site to the functional changes we observe," says Xu. Covarrubias's group is also studying propofol binding to voltage-gated sodium channels in more detail, so we'll be watching for both laboratories to uncover more clues to the mystery of anesthesia.

1. Ouyang, W., et al. 2003. *Mol. Pharmacol.* 64:373–381.
2. Wang, Y., et al. 2018. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201811993
3. Yang, E., et al. 2018. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201711924
4. Lee, S., et al. 2012. *J. Gen. Physiol.* 139:507–516.
5. Barber, A.F., et al. 2014. *Proc. Natl. Acad. Sci. USA.* 111:6726–6731.
6. Kinde, M.N., et al. 2016. *Proc. Natl. Acad. Sci. USA.* 113:13762–13767.