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Anesthesia and The Real-Time Mind

Interdisciplinary group seeks mechanism of action for inhalation agents

By Ajai Raj


Roderic Eckenhoff, MD

Fallopius of Padua, the 16th-century anatomist and physician, famously complained, “When soporifics are weak, they are useless, and when strong, they kill.” Western medicine has come a long way in the centuries since, but anesthesiologists are only beginning to understand how the drugs of their trade work at the most fundamental level.

“We don’t understand how general anesthetics work in any detail,” said Roderic Eckenhoff, MD, the Austin Lamont Professor of Anesthesiology and Critical Care at the Perelman School of Medicine at the University of Pennsylvania, in Philadelphia. “We don’t know the molecular targets that they need to engage to create their effects.”

To find out, Dr. Eckenhoff is leading a multi-institution team, along with researchers at Thomas Jefferson University, Temple University, Drexel University, Rutgers University, the University of Pittsburgh and Penn, in five connected projects. Each has the dual aim of identifying the precise binding sites where anesthetics interact with proteins in the neuronal membrane, and characterizing those interactions in detail. In addition to anesthesiologists, the group includes electrophysiologists, biophysicists, computational physicists, and structural and molecular biologists, who approach the question of how anesthetics work from different angles.

“If we can use the parable of the blind man and the elephant, we’re sort of each seeing a different bit of this problem,” Dr. Eckenhoff told *Anesthesiology News*. “But we’re then able to assemble it back to what’s really happening—what the mechanisms really are.”

Inhaled anesthetics produce a variety of effects: analgesia, immobility and amnesia, along with hypnosis and the alteration of blood pressure. Researchers know that the drugs work by regulating the activity of particular proteins in the neuronal membrane, but not which proteins are involved or how specific drugs interact with them (Figure). The likeliest

candidates are voltage-gated ion channels, which control the flow of sodium and potassium ions throughout the neuronal membrane, said Manuel Covarrubias, MD, PhD, an electrophysiologist and professor at Thomas Jefferson University's Farber Institute for Neurosciences, in Philadelphia.

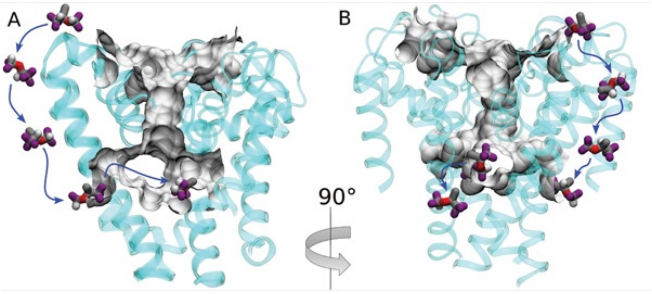
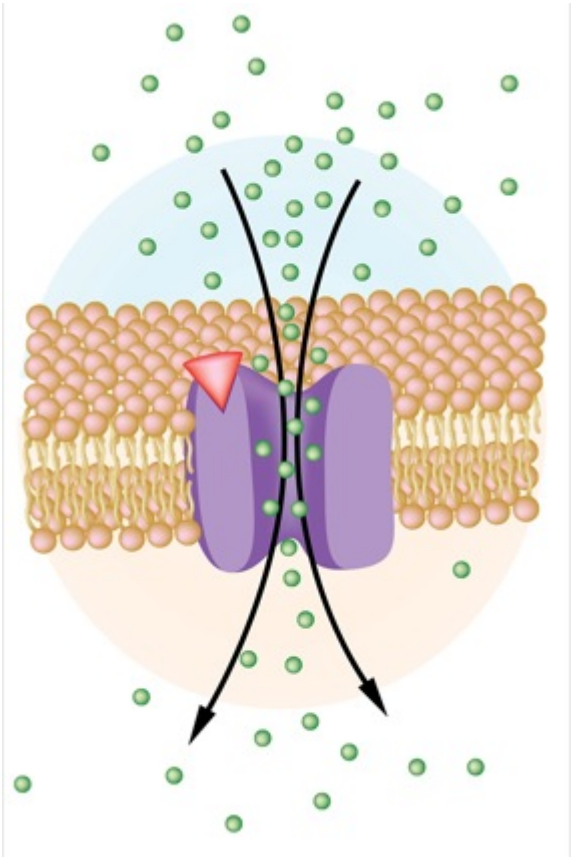


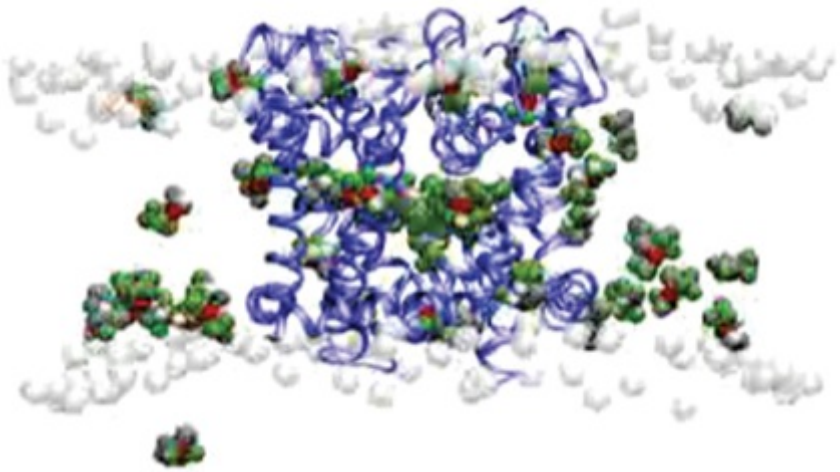
Figure. Fenestrations as the hydrophobic access pathway. (A and B) Two views of a representative isoflurane molecule traveling from bulk solution into (A) and back out of (B) the cavity via two adjacent fenestrations. Cavity and fenestrations are shown as gray surfaces for



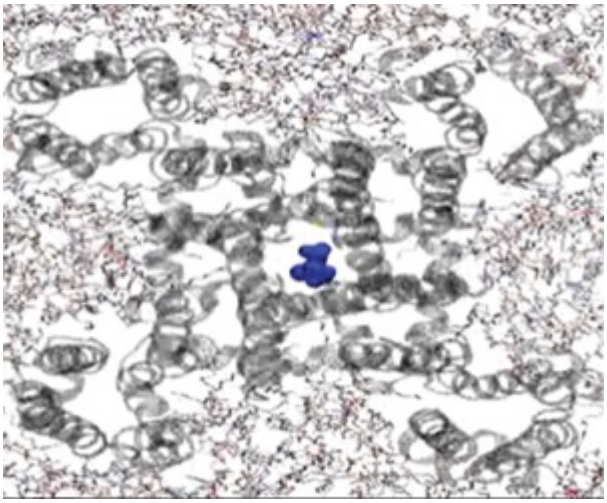
"The idea is to identify within these particular types of ion channels, which have been implicated in general anesthesia, to identify binding sites that will allow us to find the best fit for the drug, the way a hand fits in a glove," Dr. Covarrubias said. "To do that, we induce mutations in these ion channels in specific regions that we guess are the binding sites.

"Within those regions, we have identified specific residues that are possible binding sites for anesthetics," Dr. Covarrubias added. That information is crucial to creating anesthetics that target the binding sites specifically, therefore reducing or eliminating the toxicity of present drugs, such as desflurane and sevoflurane.

In addition to identifying the binding sites, the researchers also are using nuclear magnetic resonance spectroscopy to study the interaction between the anesthetics and the binding sites in real time. To complement and enhance that work, they also are creating computer simulations to model the activity of these systems over time spans as long as a microsecond—"an eon" in terms of brain activity, Dr. Eckenhoff said. Finally, they are using shallow-angle x-ray scattering and neutron spectroscopy to confirm the electrophysiologic observations and the test hypotheses derived from the computer simulations.



Movie 1. Isoflurane flooding. The NaChBac pore domain (blue ribbons) sits in the bilayer (headgroups shown as grey spheres) while isoflurane (red/green molecules) begins in the aqueous compartment above and below the bilayer and partitions first into the bilayer and then into the protein structure. Voltage sensing domains, water molecules and lipid alkyl tails are not shown in this movie but are present in the simulation.



Movie 2. Representative isoflurane trajectory through fenestration to cavity site. Bottom view of NaChBac structure (grey ribbons) surrounded by lipid molecules (stick representations). Isoflurane (blue, space-filling representation) enters from the lipid phase into the cavity through

The project, Interaction of Inhaled Anesthetics with Macromolecules, is the continuation of a collaboration that began 15 years ago. The researchers recently received $8.6 million in renewed funding for the next five years from the National Institutes of Health.

“We’re trying to get to the very basic level,” Dr. Eckenhoff said. “If you don’t know what the binding site looks like, and what atoms are needed to bind, it’s hard to do anything besides empirically alter the drug. For us to intelligently alter it—to predictably alter it—we really need to understand, at the atomic level, what’s going on.”

Electrophysiology

Traditional anesthetics are highly toxic because they are indiscriminate in how they interact with neuronal membrane proteins, Dr. Covarrubias said. “If we can identify specific interactions, then we can design more specific anesthetics,” he said. “And we believe these ion channels have those specific interactions.”

Dr. Covarrubias likens the binding sites in voltage-gated ion channels to gloves, and the anesthetics to hands. Only a few of the gloves can provide a perfect fit, by producing the desired effects of the anesthetics, without the toxicity.

To visualize the binding sites, Dr. Covarrubias uses the polymerase chain reaction to induce mutations in genes that encode specific ion channels. These genes are inserted into frog oocytes, which serve as model cells. Microscopic electrodes record where, and how strongly, the anesthetics bind to the channels.

“Sometimes by making mutations, we make that interaction better, and sometimes we make the binding worse,” he said. “So we go back and forth, to make a model of what that channel looks like.”

It’s well known that anesthetics target neurotransmitters, Dr. Covarrubias said. “We believe that that is only part of the story, that general anesthesia not only implicates the neurotransmitter receptors, but these voltage-gated ion channels as well.”

Protein Production

This project requires a wide variety of proteins, in vast quantities, in order to run the experiments— a requirement shared by x-ray crystallographers.

“It turns out that this technique demands large amounts of pure protein, so crystallographers become good at producing protein,” said Patrick Loll, PhD, a molecular biologist at the Drexel University College of Medicine, in Philadelphia. “Manufacturing proteins that are anesthetic targets will be an important part of our work going forward.”

Dr. Loll and his colleagues use genetic engineering methods to induce yeast and insect cells to produce the proteins. Once the cells have manufactured the molecules, researchers deploy a combination of affinity chromatography methods and fast protein liquid chromatography instruments to extract them from the cells.

“These instruments utilize high pressure to perform the chromatography experiment in a few minutes, so we can isolate these relatively fragile molecules quickly, before they deteriorate,” Dr. Loll said.

Once the proteins are extracted, they are analyzed using mass spectroscopy, both initially to confirm their identities, and in experiments to assess their interactions with anesthetics.

“Our long-term goal is to gain a mechanistic, molecular view of how anesthetics interact with proteins, and how this interaction gives rise to the physiological changes that we know as anesthesia,” Dr. Loll said. “That’s pretty ambitious, but we have a tremendous team working on this problem, so I’m optimistic that we’ll make great progress.”

Computational Physics

The interactions between anesthetics and these binding sites occur so quickly, and on such an infinitesimal scale, that even the most precise techniques of physical visualization are unable to render them fully. That's where computer simulations come in. Each molecule involved in the drug interactions is modeled using data derived from experimental crystallography, a method known as molecular dynamics simulation (Movies 1 and 2).

"We've got a bunch of atoms basically in a box, and they're interacting via forces we've determined by quantum chemistry," said Grace Brannigan, PhD, a computational biophysicist at Rutgers University's Camden, N.J. campus. "And we basically watch a movie of how they interact, which allows us to see how the system evolves over time."

Dr. Brannigan and her colleagues are looking at a group of neurotransmitter receptors in the neuronal membrane called cys-loop receptors. Some cys-loop receptors inhibit neuronal activity, whereas others excite this activity. Anesthetics work by negating these effects—activating cys-loop receptors that cause inhibition, and deactivating those that cause excitation.

By modeling these interactions, researchers are able to generate testable hypotheses about where anesthetics bind with cys-loop receptors, and which effects this binding produces. These hypotheses inform the physical experiments in the project, which in turn enable them to refine the computer models, in a mutually beneficial feedback loop.