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Anesthetic-Receptor Relationship Status: “It's complicated”

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How do you respond when a patient or new resident asks, “how do anesthetics work?” Do you respond with, “they inhibit neuronal activity,” or perhaps, “it's complicated. We just don't know”. Thanks to a paper in this edition of *Anesthesiology*, “Tryptophan and Cysteine Mutations in M1 Helices of $\alpha 1\beta 3\gamma 2L$ γ -Aminobutyric Acid Type A Receptors Indicate Distinct Intersubunit Sites for Four Intravenous Anesthetics and One Orphan Site,” Nourmahnad et al.¹ have enabled our field to take an enormous leap forward in our understanding of how anesthetics work.

Most general anesthetics enhance the function of GABA_A receptors, the most common neurotransmitter receptor found at inhibitory synapses in the brain.² General anesthetic molecules exist in varied shapes and sizes; therefore, each anesthetic molecule will make many interactions within a given GABA_A receptor binding site. The study by Nourmahnad et al.¹, through its thoughtful design and careful execution, beautifully demonstrates the array of interactions made by anesthetic molecules in their GABA_A receptor binding sites. The authors show that propofol, etomidate, and a barbiturate have overlapping but non-identical positions and contacts within a single binding pocket. The authors elegantly show how the pockets simultaneously accommodate molecules with different dimensions, yet form a highly selective array of bonds with each drug. The cartoon in Figure 1 of Nourmahnad et al.¹ illustrates that there are 2 kinds of propofol binding sites on the GABA_A receptor. One binding site can be shared with etomidate, and the other can be shared with a barbiturate.

There are several established ways to infer that a single residue in a protein interacts with an anesthetic molecule:

1. Mutate the residue^{*}, hoping that the critical interaction goes away:
conventional site directed mutagenesis
2. Mutate the residue^{*} to a larger amino acid that mimics an anesthetic molecule: *tryptophan scanning mutagenesis*

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RE: 16-00452 (Forman) Tryptophan and Cysteine Mutations in M1 Helices of $\alpha 1\beta 3\gamma 2L$ γ -Aminobutyric Acid Type A Receptors Indicate Distinct Inter-subunit Sites for Four Intravenous Anesthetics and One Orphan Site

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^{*}The following nomenclature is used to identify a mutation: if the 236th amino acid in the $\alpha 1$ subunit polypeptide chain is mutated from a methionine (M) to a tryptophan (W), the mutation is $\alpha 1(M236W)$.

3. Mutate the residue^{*} to a reactive residue that can be chemically modified: *Substituted Cysteine Accessibility Method (SCAM)*
4. Use a light-reactive drug that will attach to the binding site: *Photolabeling*

Nourmahnad et al.¹ set out to confirm findings from photolabeling studies suggesting that transmembrane residues $\alpha 1$ (M236) and $\beta 3$ (M227) are critical for anesthetic actions on the GABA_A receptor. Confirming these findings using methods that precisely target these residues and measure direct actions on channel function was necessary, since photolabels can erroneously label residues near but not at the desired target site. Nourmahnad et al.¹ used a modified form of the substituted-cysteine accessibility method (SCAM)³ (renamed “SCAMP”), to confirm that residues $\alpha 1$ (M236) and $\beta 3$ (M227) do indeed contact four different intravenous anesthetics. SCAMP experiments also reveal that etomidate forms contacts with $\alpha 1$ (L232) and the barbiturate, mTFD-MPAB, forms contacts with $\beta 3$ (L231).

The authors also substituted tryptophan at $\alpha 1$ (M236W) and $\beta 2$ (M286W) to mimic the effects of anesthetic binding on GABA_A receptors. In theory, the large, bulky tryptophan side chain will occupy the same space as the anesthetic molecule to exert similar effects on channel function. Nourmahnad et al.¹ show that the $\alpha 1$ (M236W) and $\beta 2$ (M286W) mutations mimic the effects of anesthetic binding on GABA_A receptors, offering further support to the hypothesis that these residues form essential interactions with anesthetics. The $\beta 3$ (M227W) and $\gamma 2$ (I242W) mutations, on the other hand, decrease sensitivity of the receptor to GABA, and the $\beta 3$ (L231W) mutation only partially mimicked the actions of anesthetics on GABA_A receptor function.

Similarly, if tryptophan occupies the anesthetic binding pocket, this mutation should occlude anesthetic binding. The $\beta 3$ (M227W) mutation reduced receptor sensitivity to some of the anesthetics examined in this study, but not all. Nourmahnad et al.¹ conclude these selective effects suggest the tryptophan substitution method is not ideal for identifying drug interactions, but is instead more useful for probing allosteric linkages to the channel gate.

Finally, Nourmahnad et al.¹ examined the role of the alpha-gamma GABA_A receptor subunit interface in anesthetic binding. They found no evidence of intravenous anesthetic binding in this part of the receptor, concluding that it is an orphan site – a site for which there is currently no known ligand. This is a particularly interesting finding since benzodiazepines exert their actions within this subunit interface. The site examined in the present study is only 44 angstroms away from the critical benzodiazepine site (equivalent to about 6 propofol molecules).

Very few recent studies on anesthetic mechanisms in GABA_A receptors are as complete as this study from Nourmahnad et al.¹ The major strength is the exhaustive examination of anesthetic sites in GABA_A receptors. Nourmahnad et al.¹ have investigated the actions of 4 drugs, on 2 residues, across 5 receptor subunit pockets, using 2 methods, compared against 2 previous datasets, yielding 160 potential comparisons. This is a deeply complex study, and the experimental design was a massive undertaking. For example, in the SCAMP experiments, the authors did not simply observe a change in modulation, they measured the rates of modification. In so doing, they uncovered a competitive interaction and directly

linked the site to drug action. Furthermore, Nourmahnad et al.¹ have not only investigated the putative site of anesthetic action examined in photolabeling studies, but they have examined all of the equivalent sites in the other GABA_A receptor subunit interfaces. The meticulous use of all 3 GABA_A receptor subunits, including the often excluded gamma subunit, combined with the thorough analysis of rate of modification, and the rigorous use of statistics reveal a more complete picture of the anesthetic binding sites than we have seen to date.

As complete as this study is, of course, it still does not provide us with the final, full story of anesthetic actions on the GABA_A receptor. Nourmahnad et al.¹ have extended our knowledge of what constitutes the walls of the propofol and etomidate binding pockets; however, there is much work to do in discovering how anesthetics stabilize and manipulate these pockets to change receptor function. While additional work is required to unequivocally confirm the exact GABA_A receptor residues that contact anesthetic molecules, this contribution from Nourmahnad et al.¹ extends our knowledge in a dramatic way.

Every drug-receptor study has the potential to define a pharmacophore for the perfect general anesthetic. These studies will always offer the hope that safer drugs with more desirable kinetics will be developed in the future. But these lofty goals are unlikely to come to fruition anytime soon. However, studies like these do two important things for anesthesiology today. First, this study, and others like it, will enhance anesthesiology as an academic discipline. Second, the findings reported in this paper will help neurobiologists, biochemists, psychiatrists, and biophysicists better understand brain function. Again, this underpins the importance of anesthesiology as an academic discipline, helping to lead discovery in translational medicine.

And finally, returning to our original question, “how do anesthetics work?” We now know a great deal more about the specific molecular targets where general anesthetics exert their actions in the brain. The findings of Nourmahad et al.¹ could even help guide the design of improved anesthetic molecules. However, elucidating how the complex microscopic interactions between anesthetic drugs and GABA_A receptors suspend consciousness, will take many more years of research.

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