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 an article in this edition of Anesthesiology, "Tryptophan and Cysteine Mutations in M1 Helices of α1β3γ2L 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.

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Most general anesthetics enhance the function of γ-aminobutyric acid type A ($GABA_A$) 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 nonidentical 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 in the study by Nourmahnad *et al.*[1] illustrates that there are two 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
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 α1(M236) and β3(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 SCAM[3] (renamed *SCAMP*: substituted cysteine modification-protection) to confirm that residues α1(M236) and β3(M227) do indeed contact four different intravenous anesthetics. SCAMP experiments also reveal that etomidate forms contacts with α1(L232), and the barbiturate, *R*-5-allyl-1-methyl-5-(*m*-trifluoromethyl-diazirinylphenyl) barbituric acid, forms contacts with β3(L231).

The authors also substituted tryptophan at α1(M236W) and β2(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 α1(M236W) and β2(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 β3(M227W) and γ2(I242W) mutations, on the other hand, decrease sensitivity of the receptor to GABA, and the β3(L231W) mutation only partially mimicked the actions of anesthetics on $GABA_A$ receptor function.

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Corresponding article on page 1144.

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*The following nomenclature is used to identify a mutation: if the 236th amino acid in the α1 subunit polypeptide chain is mutated from a methionine (M) to a tryptophan (W), the mutation is α1(M236W).

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Similarly, if tryptophan occupies the anesthetic binding pocket, this mutation should occlude anesthetic binding. The β3(M227W) mutation reduced receptor sensitivity to some of the anesthetics examined in this study but not all. Nourmahnad *et al.*[1] conclude that these selective effects suggest that 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 α–γ $GABA_A$ 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 current study is only 44 Å away from the critical benzodiazepine site (equivalent to about six propofol molecules).

Very few recent studies on anesthetic mechanisms in $GABA_A$ receptors are as complete as the study by Nourmahnad *et al.*[1] The major strength is the exhaustive examination of anesthetic sites in $GABA_A$ receptors. Nourmahnad *et al.*[1] investigated the actions of four drugs on two residues across five receptor subunit pockets using two methods compared against two 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; instead, they measured the rates of modification. In doing so, they uncovered a competitive interaction and directly linked the site to drug action. Furthermore, Nourmahnad *et al.*[1] not only investigated the putative site of anesthetic action examined in photolabeling studies but also examined all of the equivalent sites in the other $GABA_A$ receptor subunit interfaces. The meticulous use of all three $GABA_A$ receptor subunits, including the often excluded γ 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] 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 article 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.

Finally, we return 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.

Competing Interests

The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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