Gone Fishing…

Roderic G. Eckenhoff, M.D.

CURRENTLY used general anesthetics are almost invariably effective, but nagging side effects, both short (*e.g.*, cardiac depression) and long (*e.g.*, neurotoxicity) term, have reawakened the call for new drugs. After all, it has been almost 25 yr since the last inhalational anesthetic (sevoflurane) was introduced and almost 30 yr since the last IV drug (propofol) was released in the United States. There are two general approaches for discovering new drugs. The first is tweaking existing drugs to either enhance the desired effect, or to mitigate some undesired effect. For example, the principle side effect of etomidate was adrenocortical suppression, which was shown to be due to a very high affinity interaction with the 11-β hydroxylase enzyme. This was effectively mitigated by either changing the molecule to remove the interaction, or through addition of an autodestruct moiety (*e.g.*, "softening" the molecule to provide for faster elimination).[1,2] Interestingly, neither change to the etomidate molecule significantly diminished hypnotic activity, implying that the desired effect may actually be the pharmacologically less specific one. This also suggests that, while the molecular targets underlying side effects are sometimes known, or at least knowable, the desired effect targets remain enigmatic.

The second general approach to drug development is in many ways the more exciting, because despite having a much lower success rate, it holds the promise of discovering truly new drugs. Used by Yang *et al.*[3] in this issue of ANESTHESIOLOGY, "high throughput screening" examines large libraries of compounds for a desired activity. But it usually starts with a molecular target. What to use for general anesthesia?

The γ-aminobutyric acid type A ($GABA_A$) receptor? The tandem-pore K-channels? The *N*-methyl-D-aspartate receptor? The mitochondrial respiratory complexes? All of them? The fact that each of these targets has been shown to contribute to the hypnotic endpoint of many general anesthetics raises a particular problem for high throughput screening.

"…the first to employ [the Zebrafish] model to discover new anesthetics, and the results are promising."

Typically, the chosen target gets incorporated into a miniaturized, robotically driven assay system, making possible screens of millions of compounds for activity (either experimentally or computationally) in a few days. Further medicinal chemistry refines the screen "hits" to optimize activity before ever being given to an animal. Although failures at this stage are the rule, exceptions, such as some cancer and anti–human immunodeficiency virus drugs, have resulted. Several years ago, we tried the high throughput screen approach by adopting a surrogate molecular target, apoferritin.[4] Why apoferritin? Because, quite remarkably, it bound many general anesthetics in an Overton–Meyer manner, it is inexpensive, and a simple binding assay was available. Less than 1% of almost 400,000 compounds bound as effectively as propofol, but when subjected to secondary assays of, for example, γ-aminobutyric acid–mediated activity, there was no correlation, despite the fact that we had considered apoferritin to be a surrogate model of the $GABA_A$ receptor. However, at least two were reversible immobilizers in rodents. Ultimately, it's not clear if this screening result is better than random choice from the library. So, do we abandon the screening approach for finding new anesthetics?

Eventually, drugs have to work in humans and/or animals, so in the absence of a validated target, why not start in the animal? The obvious answer is that screening millions of compounds using a typical anesthetic phenotypic assay (*e.g.*, immobility) would take decades—unless you can "miniaturize" the assay and do hundreds of animals simultaneously. Enter the zebrafish (*Danio rerio*). Not only a favorite pet, but also a favorite animal model of the developmental biologists and geneticists. Zebrafish are extremely well characterized; thousands of genetically-modified forms, as well as reagents, are readily available. Husbandry is easy, and most importantly, the larvae display conserved motor responses to various forms of noxious stimulation (light, vibration, and electricity, among others). The larvae fit nicely into the wells

Image: ©ThinkStock.

Corresponding article on page 459.

Accepted for publication May 17, 2018. From the Department of Anesthesiology and Critical Care, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania.

<zdoi;10.1097/ALN.0000000000002328>

of a 96-well plate, automated movement detectors and software are readily available, and *voila*: a (semi) high throughput system for testing chemical induced-immobility. Yang *et al.*[3] are the first to employ this model to discover new anesthetics, and the results are promising.

These authors used loss of the photomotor response in 6- to 7-day postfertilization larvae as the anesthetic endpoint. The photomotor response is a stereotypical movement after a brief flash of light, and is highly amenable to automation. The authors first validated this endpoint in zebrafish larvae using a battery of known compounds and found the activities and potencies to precisely match those of tadpoles and mammals. They then screened a small, but diverse, library of 374 compounds in 96-well format, each plate containing positive etomidate controls. They discovered two compounds that reversibly inhibited the photomotor response at 10 to 20 μM; more detailed dose-response experiments determined the EC_{50} to be about 10 μM for each.

Such "hits" are then typically tweaked using medicinal chemistry to produce more effective "probe" compounds, a step not yet taken by Yang *et al.* In contrast to the usual high throughput screen, which starts with the mechanism and ends at the phenotype, Yang *et al.* did the opposite. So to get at underlying mechanisms, the investigators applied these two compounds in ion channel studies. They found only weak activity at the canonical anesthetic targets, including the $GABA_A$ receptor, *N*-methyl-D-aspartate, and nicotinic acetylcholine receptors. Perhaps disappointing to those wed to these targets, this result should be considered a very exciting observation. When coupled with the fact that these compounds are completely novel chemotypes, this implies the existence of other, as yet untapped, pathways to anesthesia (assuming that is what loss of the photomotor response here represents).

The anesthetic phenotype in the zebrafish is only a stepping stone; the next step is translation to an intact mammal. But it is important to understand that the available mass of compounds in chemical libraries is typically insufficient to support rigorous efficacy studies, even in rodents. Yang *et al.* had only enough material to do a few rat studies, so these should be viewed as only exploratory examples of the approach rather than definitive results, *per se*. For example, the lack of immobilizing effect of one of the two hits in only two rats, in the absence of blood levels, could easily be a false negative. But before one synthesizes more of these two compounds, which are, after all, not as potent as either etomidate or propofol, an alternate plan might be to screen much larger libraries, in order to reveal more efficacious reversible immobilizers that exist in chemical space. As succinctly stated in the abstract: "The variety of chemotypes producing hypnosis is likely much larger than currently known." Amen.

Competing Interests

The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article.

Correspondence

Address correspondence to Dr. Eckenhoff: reckenho@pennmedicine.upenn.edu

References

1. Shanmugasundararam S, Zhou X, Neunzig J, Bernhardt R, Cotten JF, Ge R, Miller KW, Raines DE: Carboetomidate: An analog of etomidate that interacts weakly with 11β-hydroxylase. Anesth Analg 2013; 116:1249–56
2. Ge R, Pejo E, Husain SS, Cotten JF, Raines DE: Electroencephalographic and hypnotic recoveries after brief and prolonged infusions of etomidate and optimized soft etomidate analogs. Anesthesiology 2012; 117:1037–43
3. Yang X, Jounaidi Y, Dai JB, Marte-Oquendo F, Halpin ES, Brown LE, Trilles R, Xu W, Daigle R, Yu B, Schaus SE, Porco JA Jr., Forman SA: High-throughput screening in larval zebrafish identifies novel potent sedative-hypnotics. Anesthesiology 2018; 129:459–76
4. McKinstry-Wu AR, Bu W, Rai G, Lea WA, Weiser BP, Liang DF, Simeonov A, Jadhav A, Maloney DJ, Eckenhoff RG: Discovery of a novel general anesthetic chemotype using high-throughput screening. Anesthesiology 2015; 122:325–33