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Seeing the Light

Protein Theories of General Anesthesia

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Do General Anaesthetics Act by Competitive Binding to Specific Receptors? By Nicholas P. Franks, William R. Lieb. Nature 1984; 310:599–601. Reprinted with permission.

Most proteins are insensitive to the presence of general anaesthetics at concentrations which induce anaesthesia, while some are inhibited by some agents but not others. Here we show that, over a 100,000-fold range of potencies, the activity of a pure soluble protein (firefly luciferase) can be inhibited by 50% at anaesthetic concentrations which are essentially identical to those which anaesthetize animals. This identity holds for inhalational agents (such as halothane, methoxyflurane and chloroform), aliphatic and aromatic alcohols, ketones, ethers and alkanes. This finding is all the more striking in view of the fact that the inhibition is shown to be competitive in nature, with anaesthetic molecules competing with the substrate (luciferin) molecules for binding to the protein. We show that the anaesthetic-binding site can accommodate only one large, but more than one small, anaesthetic molecule. The obvious mechanism suggested by our results is that general anaesthetics, despite their chemical and structural diversity, act by competing with endogenous ligands for binding to specific receptors.

WE were pleased to learn that our article[1] on the effects of general anesthetics on the firefly luciferase enzyme has been selected as a "classic paper." It was not always so. Some time after the results were first presented (at the Third International Conference on Molecular and Cellular Mechanisms of Anesthesia, held at Calgary, Alberta, Canada, from June 13 to 15, 1984), a commentary[2] on the meeting described our results as "an interesting departure from the mainstream" and concluded, rather grudgingly we thought, that "it appears, in principle, that protein sites cannot be ruled out." Despite this being the faintest of faint praise, for us, this was real progress. Since our first publication[3] together, we had been arguing that the conventional view that general anesthetics acted by disrupting lipid bilayers was seriously flawed, and we still believed we were regarded by the community as antisocial dissidents.The luciferase article was a significant milestone in our own research because it provided the strongest evidence yet that the idea that general anesthetics might act by binding directly to sensitive proteins was plausible, if not probable. All our previous work had been directed at pointing out what we perceived to be the shortcomings of the various lipid theories that had been in vogue, and the luciferase work gave us a positive lead that shaped our research for several years.

The idea of working on the effects of general anesthetics on a bioluminescent system was certainly not original. In fact, it was while writing a review[4] on anesthetic mechanisms that we realized how much excellent work had been done in this area, particularly on bioluminescent bacteria, that dated back to the 1930s. (It was during these early studies that the so-called "pressure-reversal" of general anesthesia was first discovered, but that is another story.) What this body of research persuasively showed was that the light emitted by bioluminescent bacteria was depressed by anesthetics in a way that correlated with their potencies in whole animals. Moreover, although work on cell-free systems was extremely limited, there was one study,[5] using a crude cell-free extract from fireflies, that indicated that the correlation might have its origins at the molecular level, rather than at the level of the whole organism. We decided to pursue this possibility.

Up until 1983 the only experimental system that we had worked on together was the lipid bilayer. As one of our more acerbic colleagues put it, "the only thing you guys have ever anesthetized is an egg yolk." The idea of working on an enzyme was, therefore, a step into the unknown for us. We first had to learn how to purify the firefly luciferase enzyme so that we could be sure it was free of any traces of other cell components, particularly

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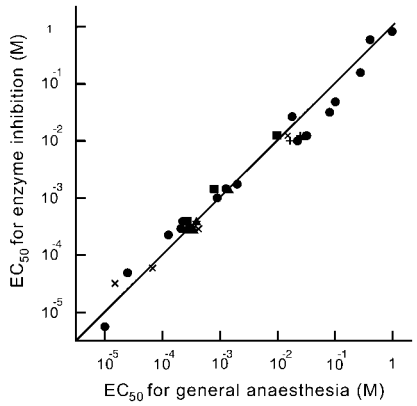

Fig. 1. The concentrations of a diverse range of general anesthetics needed to half-inhibit the firefly luciferase enzyme are essentially identical to those that induce general anesthesia in animals, over a 100,000-fold range of concentrations. The *line* is the line of identity. ■ = man; x = mouse; + = newt; ● = tadpole; ▲ = goldfish. The data were taken from Franks and Lieb.[1]

lipids. We were greatly aided by a wonderfully elegant purification protocol[6] that yielded showers of crystalline protein after only a couple of days' work. We then built a rapid-mixing system that injected adenosine triphosphate (which triggered the reaction) into a small glass vial containing all the other components and from which the resulting flash of light could be detected using a sensitive photomultiplier.

Within weeks, we were getting reliable and reproducible results. We soon realized that not only was the firefly enzyme extremely sensitive to anesthetics but that the correlation between its sensitivity and whole animal potency was going to be remarkable. It would provide a serious challenge to the famous Meyer-Overton correlation between animal potency and lipid solubility that had been such a dominant influence in the field. During the months between May 1983 and January 1984, we accumulated data on a disparate group of anesthetics, including ethers, alkanes, alcohols, and ketones. The correlation between enzyme inhibition and anesthesia in animals held over a 100,000-fold range of potencies (fig. 1). Moreover, we showed that the mechanism of inhibition was competitive in nature, with all of the anesthetics inhibiting the enzyme by simply preventing the binding of one of the natural substrates, firefly luciferin (a heterocyclic molecule that, when raised to an excited state, is responsible for emitting the photon). The data were consistent with all of the anesthetics binding to a common site. We were able to estimate the size of this binding "pocket" on luciferase as being approximately 250 ml/mol because of the way in which the stoichiometry of binding changed with anesthetic size.

The competitive nature of the inhibition was important because it allowed us to readily extrapolate our results to other proteins—had the inhibition been due to some obscure perturbation of the catalytic machinery peculiar to light-emitting enzymes, it would have been hard to argue that such a mechanism might apply more generally. However, competitive binding shows that the protein provides a suitable environment within which anesthetics can bind. If such an environment exists on the luciferase enzyme, then why should a similar environment not exist on one (or several) proteins or ion channels in the central nervous system?

The possible importance of direct anesthetic interactions with proteins had, of course, been discussed before, but often on theoretical rather than experimental grounds. The luciferase work brought together several elements of the argument—sensitive inhibition of function, an excellent correlation with potency, and a simple and generalizable mechanism—based on good experimental data. This, coupled with the growing realization that something really was lacking in the old lipid theories, combined to make this article a significant milestone.

Milestones, however, are just markers and do not necessarily define the route ahead, and it is impossible for us to say whether this work was a major influence on the field of anesthetic mechanisms. Looking back 20 yr at our notes and correspondence, it is obvious that the climate then was radically different, but this is probably true of any field. We will leave it to others to say whether they were influenced, but it certainly influenced our own work. Despite several years of arguing the importance of anesthetic/protein interactions, we were in a more or less permanent state of anxiety about whether we were on the right track. The luciferase data convinced and reassured us that we probably were, and we completed a series of studies culminating with the crystallographic demonstration of an anesthetic binding site on the enzyme.[7] Throughout this time, we have followed one simple hypothesis—that anesthetics act at a relatively small number of sensitive protein targets[8]—and we have used strategies aimed at identifying these targets. Satisfyingly, several other groups around the world have been doing the same, and progress during the past 5 yr or so has been remarkable. The demonstration that specific mutations to a particular receptor subunit (from the γ-aminobutyric acid type A receptor) can remove, or at least greatly diminish, the anesthetic sensitivity of not only the receptor[9,10] but also a genetically modified animal[11] is something that few would have thought likely 20 yr ago.

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