

REPORTS OF SCIENTIFIC MEETINGS

David E. Longnecker, M.D., Editor

The Third International Conference on Molecular and Cellular Mechanisms of Anesthesia: A Report

The Third International Conference on Molecular and Cellular Mechanisms of Anesthesia* was held at the University of Calgary in Alberta, Canada, from June 13–15, 1984. Two prior meetings in this series were chaired by B. Raymond Fink in Seattle in 1974 and 1979. The co-chairmen for the Third International Conference, Sheldon Roth (Calgary) and Keith Miller (Harvard), maintained Dr. Fink's tradition of inviting distinguished investigators from the physical and biologic sciences to articulate their views on anesthetic action in a series of research lectures, panel discussions, and poster sessions. These conferences have done a great deal to promote scholarly exchange, debate, and research in the complex field of anesthetic mechanisms. For this, the anesthesia world is much indebted to Dr. Fink, and a banquet acknowledging his contributions followed the first day's activities.

The opening symposium, devoted to anesthetic actions on Voltage-Gated Ion Conductances, was chaired by Gary Strichartz (Harvard). The morning began with a historic overview of research on local anesthetic mechanisms from Murdoch Ritchie (Yale). Although this year marks the hundredth anniversary of Freud's description of local anesthesia using cocaine, the current understanding of local anesthetic actions on excitable membranes is scarcely 20 years old. That local anesthetics interfere with neuronal excitation predominantly by interacting with voltage-dependent sodium channels is no longer in dispute, but important questions remain: What is the active species of a local anesthetic? Are the sodium channels the only functionally significant local anesthetic targets, or are other ion channels also affected? What are the molecular interactions by which local anesthetics affect sodium channels? Do they bind more readily to open or closed states of the channel? Does the differential sensitivity of neuronal fiber types to local anesthetics reflect physiologic differences among fiber types, or can it be explained on a pharmacokinetic basis? What kinds of dramatically new agents (for example, drugs offering a reversible nerve block of several weeks' duration) may we reasonably expect in the future? These questions are being explored, Dr. Ritchie said, largely within the context of the Modulated Receptor Hypothesis, proposed in 1977 by Bertil Hille. Briefly, this theory holds that ion channels undergo transitions among (at least) three states: closed (resting), open, and inactivated. Anesthetics may interact with any or all of these states. The model thus generates strong predictions that may be tested experimentally.

One such prediction, that local anesthetics act by stabilizing an inactive channel conformation, derived apparent support from studies by Bruce Bean (Iowa) on the antiarrhythmic action of lidocaine. He presented evidence from voltage-clamped Purkinje fibers that, at concentrations far lower than those required to block impulse conduction in the nerve, lidocaine produces a use-dependent block that arises from binding to inactivated sodium channels. In principle, however, use-dependent block could arise from drug binding either to inactivated states of the channel, as originally postulated on the Modulated Receptor Hypothesis, or to activated or open channels. Dr. Strichartz presented several lines of evidence indicating that in nerve, it is unnecessary to postulate local anesthetic binding to inactivated channels. For example, in nerve fibers treated with specific toxins that "remove" the normal inactivation process, use-dependent block was not affected. Thus, although a full account is not yet possible, it is clear that no single mechanism can account for the various effects exerted by local anesthetics at different tissue sites.

Jay Yeh (Northwestern) presented an analysis of local anesthetic actions on sodium channels in neuroblastoma cells using a new electrophysiologic method known as "patch-clamp" or "single-channel" recording. This powerful technique permits direct observation of the behavior of individual ion channels. The effects of anesthetics on such fundamental channel characteristics as conductance and mean open time thus can be measured directly rather than inferred. Local anesthetics have been shown to exert complex effects on the kinetics of transitions between open and closed states of ion channels. For example, Dr. Yeh found that some agents induced a state in which the channels appeared to "flicker" rapidly between open and closed states, while others merely shortened the duration of the open state. Analysis of these kinetic patterns is yielding important information on the physical interaction between anesthetic and channel, but a full description is not yet available. Max Willow (University of Washington) reported experiments examining the effects of local anesthetics and anticonvulsants on sodium channels from mammalian brain "synaptosomes," which are the pinched-off terminals of neurons. It has been demonstrated that sodium channels are the biologically important target for a number of potent neurotoxins found in nature. Dr. Willow and his collaborators used batrachotoxin, a frog neurotoxin, which causes persistent activation of sodium channels, as a probe to characterize the interaction of drugs with these channels. They found that local anesthetics, diphenylhydantoin and carbamazepine, all inhibited batrachotoxin binding and that this inhibition was mediated by an allosteric (noncompetitive) mechanism. These experiments, together with others using similar toxins, have revealed that

* Proceedings to be published by Plenum Press.

the sodium channel macromolecule exhibits at least three functionally distinct drug- and toxin-binding sites.

Denis Haydon (Cambridge) summarized work from his laboratory on the effects of general anesthetics and other nonionic lipophilic agents (hydrocarbons) on sodium currents in voltage-clamped squid axon. These agents were shown to produce small shifts in the sodium channel population favoring both closed and inactivated states. Moreover, the thickness of the axonal membrane, assessed by electrical capacitance measurements, was increased by the hydrocarbon anesthetics. It was proposed that this membrane thickening could, in itself, explain the observed changes in sodium channel function, without postulating specific interactions of agents with the channels themselves. Despite the elegance of these studies, they illustrated how much more work must be done with general anesthetics, if advances like those made with local anesthetics are to be forthcoming.

A symposium on Chemically-Gated Ion Conductances examined the effects of anesthetics on channels that are activated by neurotransmitters rather than voltage. Both an excitatory neurotransmitter receptor-channel complex, the acetylcholine receptor (AChR), and an inhibitory one, the gamma-amino butyric acid (GABA) receptor, were featured, because it is not yet known whether the anesthetic state results primarily from perturbations of excitatory, inhibitory, or both types of synaptic transmission. In a lucid introduction, Palmer Taylor (San Diego) argued that the nicotinic acetylcholine receptor constitutes a well-defined system (the AChR is, by a wide margin, the most completely characterized membrane receptor) in which to study anesthetic actions on postsynaptic excitatory transmission. Each AChR-channel complex is an oligomer of five transmembrane polypeptide chains that together contain two agonist binding sites, probably a single local anesthetic site, and a central channel through which ions may cross the membrane. The affinity of the AChR for its ligand ACh is altered by volatile and (most) local anesthetics (which enhance affinity) and by barbiturates (which reduce affinity). Although a specific, saturable, allosteric site produces the agonist affinity change noted with local anesthetics, less is known about the mechanism by which general anesthetics produce enhanced affinity for nicotinic agonists. Using mammalian skeletal muscle cells as a source of AChR, Dr. Taylor employs fluorescent toxins to measure the molecular distances between agonist-binding sites and various allosteric binding sites. The method promises to reveal structural correlates of the functional changes in receptor properties produced by anesthetics. In particular, the high-affinity state of the receptor is associated with a "desensitized" ion channel. Desensitization of ACh-gated channels is in certain respects analogous to inactivation of voltage-gated channels: both processes terminate membrane excitation; both are enhanced by anesthetics.

The mechanisms by which a variety of local anesthetics influence AChR behavior were investigated by Jonathan Cohen (Washington University), who presented radioligand binding experiments carried out in parallel with ion flux studies. Using AChR-rich membranes from the Torpedo (a marine ray) electric organ he found that different local anesthetics vary greatly in potency at the allosteric binding site (through which AChR are shifted to the high-affinity, desensitized state). Since

the allosteric linkage between drug- and ACh-binding sites conceivably could be mediated either through the protein structure itself or its surrounding lipid, the mechanism by which anesthetics desensitize AChR membranes may be quite complex.

Keith Miller (Harvard) summarized his findings on the effects of volatile and barbiturate anesthetics on both the structure and function of Torpedo AChR in their lipid environment. In conjunction with classical binding studies, Dr. Miller's group has measured membrane lipid order, as well as ion flux through the AChR-channels. At clinical concentrations, volatile anesthetics dramatically increase ACh binding by stabilizing a high-affinity receptor conformation, and in parallel, disorder the membrane lipid bilayer. Barbiturates, on the other hand, bind at low concentrations to an allosteric site on the receptor, causing inhibition of ACh binding. At higher concentrations, they increase ACh binding and disorder the membrane in a manner reminiscent of the volatile anesthetics. Thus, the effects of these drugs on the binding of ACh to its receptor appear to be mediated through both direct (protein) and indirect (lipid) mechanisms. Their effect on the resulting ion flux is to decrease it, consistent with the concept of enhanced desensitization.

Peter Gage (Australian National University) presented a patch-clamp analysis of anesthetic actions on the AChR, along lines similar to those described for the voltage-dependent sodium channels. Dr. Gage's evidence favored the concept that volatile and barbiturate anesthetics decrease the average "open time" of AChR but do not actually block the open state. He also has examined anesthetic effects on the GABA-activated chloride channel, which mediates inhibitory synaptic transmission at many sites throughout the cerebral cortex. Remarkably, the same anesthetics that decrease AChR channel open time (reducing excitation) increase GABA receptor channel open time (enhancing inhibition). Richard Olsen (UCLA) followed with biochemical experiments on modulation of GABAergic function by barbiturates. The agents were shown to enhance both the binding of GABA to its receptor and the resulting chloride ion flux. Thus, although a detailed alignment of measurements derived from biochemical and electrophysiologic experiments is not yet feasible, it is clear that these disparate approaches are rapidly converging toward a common view of receptor-channel behavior in the presence of anesthetics. From both kinds of studies, an apparent symmetry is emerging: that general anesthetics interfere with excitatory synaptic transmission and facilitate inhibitory synaptic transmission. It remains to be seen whether this concept will be strengthened further by studies of a wide variety of channel types.

A session entitled Biophysical Mechanisms, chaired by James Trudell (Stanford), focussed on the molecular interactions between anesthetics, interactions that form the basis of the lipid-oriented hypotheses of general anesthetic action. James Metcalfe (Cambridge), known for his pioneering work on anesthetic-induced lipid "fluidization," reviewed the myriad effects of anesthetics on the orderly structure of lipid membranes. Given this variety, he concluded that a unitary mechanism, in which all agents are postulated to act by the same lipid perturbation, is less likely than one that is "degenerate," in the sense that different lipid perturbations could result in

the same functional endpoint. Sid Simon (Duke) provided x-ray diffraction evidence that could support such a "degenerate" model, finding that only agents with strong dipoles (*i.e.*, alcohols) induce an "interdigitated" lipid gel phase; others do not. Moreover, neutron diffraction and NMR data from Steven White (Irvine) revealed that the small volatile hydrocarbons (which lack strong dipoles) dwell in regions of the bilayer that the strongly dipolar agents find relatively inaccessible. Taken together, these studies mean that part of the diversity in molecular interactions may be accounted for by subtle features in anesthetic structure. But some of that diversity resides in the structure of the lipid itself. For example, Ian Smith (Ottawa) demonstrated that the ordering/disordering effects of anesthetics depend on the phospholipid species, the acyl chain lengths, and the position in the bilayer from which measurements are made. In sum, recent refinements in lipid-based theories have come from a deepening, rather than a broadening, of our understanding of anesthetic-lipid interaction. Nick Franks and William Lieb (London) described evidence in favor of an alternative to lipid-based hypotheses, proposing that anesthetics act within the hydrophobic domains of proteins. Luciferase, the enzyme that catalyzes a light-emitting chemical reaction in the common firefly, was shown to be competitively inhibited by a structurally diverse group of anesthetics. The purified (lipid-free) enzyme was inhibited at concentrations of the anesthetics essentially identical to those that produce anesthesia, over a 100,000-fold range of potencies. Thus, it appears that a pure protein target site could, in principle, account for the extraordinary pharmacologic profile, exhibited by virtually all general anesthetics, known as the Meyer-Overton rule. This rule has traditionally been interpreted to mean that anesthetics must act within a lipid domain.

Whether anesthetics act at lipid or protein sites within the cell membrane, it is generally agreed that they ultimately must alter the fundamental processes underlying neuronal excitation. These processes have traditionally been separated into two categories, the voltage-gated and the chemically gated ion conductances, as reflected in the titles of the first two symposia. In that conceptual framework, a given membrane channel was operated either by voltage or a chemical ligand—but not both. In recent years, however, it has become clear that a third category exists. For example, calcium-activated potassium channels, which are important in modulating the integrated activity of neurons, are governed by both voltage and intracellular calcium ions. In the final symposium, Cellular and Synaptic Actions, the hypothesis that anesthetics enhance inhibition by altering the behavior of potassium channels was explored, and additional studies on the GABA-activated chloride channel were presented. Currents carried by potassium, like those carried by chloride ions, typically hyperpolarize and hence inhibit neuronal membranes. Kresimir Krnjevic (McGill), who some 10 years ago proposed that general anesthetics might activate a calcium-dependent potassium conductance by releasing calcium from intracellular sites, chaired the session and reviewed the major lines of evidence. Mary Morris (Toronto) followed with intriguing preliminary evidence, using the recently introduced fluorescent calcium indicator quin-2,

that barbiturates elevate intracellular free calcium, lending support to Krnjevic's hypothesis. Peter Carlen (Toronto) carried the argument a step further, reporting provocative studies of the effects of anesthetics on the afterhyperpolarization that characteristically follows action potentials in many central neurons and is involved in modulation of their input-output characteristics (*e.g.*, regulation of repetitive or "bursting" activity). Barbiturates and ethanol both prolonged the afterhyperpolarization, and this was attributed to enhancement of a calcium-dependent potassium conductance. The effect was observed at lower anesthetic concentrations than those at which effects on GABA-activated chloride channels could be measured. Robert MacDonald (University of Michigan) described yet a third possible inhibitory mechanism for barbiturates: interference with the presynaptic release of (excitatory) neurotransmitters. In studies on cultured spinal neurons, his group has shown an apparent reduction by barbiturates of presynaptic calcium entry, with an associated decrease in transmitter release. Enhancement of the inhibitory postsynaptic action of GABA, as well as reduction of the excitatory postsynaptic action of glutamate, also were demonstrated. David Owen (NIH), reporting experiments using both patch-clamp and classical voltage-clamp techniques on a brain slice preparation, confirmed the barbiturate potentiation of GABA action. Remarkably, it was further shown that these agents may act directly to open chloride channels in the absence of GABA. agents may act directly to open chloride channels in the absence of GABA.

Beyond these principal events, a number of "minisymposia" focused on various specialized topics, including Pressure Reversal of Anesthesia, Metabolism and Toxicity of Anesthetics, and Malignant Hyperthermia. In the final afternoon of the conference, participants enjoyed an informal session ("Current Theories of the Molecular Basis of General Anesthetics") in which the participants exchanged lively debate, scientific anecdotes and well-informed speculation. Truly, in its total information flux, the Third International Conference opened a wide variety of new channels. And yet, for those of us who daily cast the anesthetic spell, "the mystery remains intact."¹

LEONARD L. FIRESTONE, M.D.

ROGER S. BRETT, M.D., PH.D.

*Departments of Anesthesia and Pharmacology
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts*

*Department of Anesthesiology
University Hospital
SUNY at Stony Brook
Stony Brook, New York*

Reference

1. Nabokov V. *Poems and Problems*. New York, McGraw Hill, 1970.