

◆ EDITORIAL VIEWS

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Double-edged Swords

Volatile Anesthetics Both Enhance and Inhibit Ligand-gated Ion Channels

TWO articles appear in this issue of ANESTHESIOLOGY that examine the abilities of volatile anesthetics to enhance transmitter-stimulated opening of ligand-gated ion channels and to block the flow of ions through these channels. The first, by Raines and Zachariah,¹ reports that isoflurane can enhance the apparent affinity of acetylcholine for the muscle nicotinic acetylcholine receptor; this contrasts with most previous studies that have emphasized the ability of volatile anesthetics to block the flow of ions through the nicotinic receptor channel. The second article, by Banks and Pearce,² provides pharmacologic evidence that the blocking and enhancing actions of volatile anesthetics on synaptic responses mediated by γ -aminobutyric acid type A (GABA_A) receptors occur by means of actions at distinct sites.

The muscle nicotinic receptor is a member of an extended gene family of ligand-gated ion channels, which also contains the GABA_A receptor, the neuronal nicotinic receptor, the glycine receptor, and the 5-HT₃ receptor. The muscle nicotinic receptor, especially the form found at the *Torpedo* electric organ, has long been the workhorse for structural and detailed mechanistic studies of members of the ligand-gated ion channel family. This is because the *Torpedo* nicotinic receptor can easily be purified in large quantity. *Torpedo* receptors thus have been used in photoaffinity labeling studies to identify the primary structure for a variety of drug binding sites. Additionally, three-dimensional images of the *Torpedo* receptor have been obtained,³ providing a model for the tertiary structure of ligand-gated ion channels. The *Torpedo* nicotinic receptor thus would seem to be a good

model in which to study volatile anesthetic binding sites. There has, however, been significant reservation about using the nicotinic receptor as a model because the predominant effect of volatile anesthetics is to block, rather than enhance, nicotinic receptor currents. In contrast, the relevant effect of volatile anesthetics on GABA_A receptors (which are thought to be a major target for anesthetics in the brain) is likely to be enhancement of function. The demonstration by Raines and Zachariah¹ that volatile anesthetics can enhance the effect of acetylcholine on muscle-type nicotinic receptors, increases the usefulness of the *Torpedo* receptor as a model for understanding the structural basis for the actions of volatile anesthetics on ligand-gated ion channels.

The idea that anesthetics can act on ligand-gated ion channels to potentiate the effects of neurotransmitters and block transmitter-elicited currents is not new. Akaike *et al.*⁴ first observed that when pentobarbital was suddenly removed from the fluid around a cell, there was a transient increase in GABA_A current, as though inhibition caused by the barbiturate was removed when the drug was removed. Later work reported block of GABA_A receptors by high concentrations of volatile anesthetics, propofol, and steroid anesthetics. In the case of the GABA_A receptor, potentiation is seen at lower concentrations of anesthetics than block, for all of these drugs. Even previously, it was reported that alcohols, especially ethanol, could potentiate the response of the muscle nicotinic receptor to low concentrations of acetylcholine and reduce the response to high concentrations.⁵ Longer-chain alcohols, such as octanol, had such strong blocking activity that potentiation could not be seen. Subsequent work with nicotinic receptors has provided evidence that the blocking action of alcohols and isoflurane is associated with specific amino acids in the portion of the receptor protein that lines the ion channel.⁶ It is interesting that the particular mutations that have strong effects on the blocking activity of alcohols have minimal effects on the ability of ethanol to potentiate responses (S. Forman, oral communication, August, 1998). This observation indicates that the potentiating and blocking effects of ethanol have different structural requirements in the receptor protein, suggesting that the binding sites are different.

The article by Raines and Zachariah¹ uses an indirect but effective approach to disentangle the actions of isoflurane on the *Torpedo* nicotinic receptor. A fluorescence assay is used

This Editorial View accompanies the following articles: Banks MI, Pearce RA: Dual actions of volatile anesthetics on GABA_A IPSCs: Dissociation of blocking and prolonging effects. ANESTHESIOLOGY 1999; 90:120-34; and Raines D, Zachariah VT: Isoflurane increases the apparent agonist affinity of the nicotinic acetylcholine receptor. ANESTHESIOLOGY 1999; 90:135-46.

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to determine the rate of a relatively slow process: the development of a high-affinity, desensitized state of the receptor. Study of desensitization avoids the complication that ion fluxes are blocked by isoflurane. This process also is sufficiently slow that the maximum rate of desensitization can be directly measured. In this case, it is straightforward to demonstrate that the maximum rate is unchanged by isoflurane, whereas the concentration dependence of the rate is shifted to lower concentrations of acetylcholine. Hence, the *apparent* dissociation constant for acetylcholine must have changed. At this point, some ambiguity enters the interpretation. The authors assume that desensitization proceeds largely from the open-channel state of the receptor, and indeed the apparent dissociation constant for desensitization (in the absence of isoflurane) corresponds well to that for activation. However, the apparent dissociation constant includes terms related to binding and additional terms related to the ratio of the channel opening and closing rates. Hence, it is not clear whether the true binding affinity is altered or whether isoflurane enhances the probability that a channel will be open. Some evidence is presented that isoflurane does not affect the dissociation rate for a fluorescent partial agonist from one of the acetylcholine binding sites on the receptor, which is consistent with idea that binding *per se* is not altered. Studies of the action of ethanol on nicotinic receptors using partial agonists have also provided evidence that it potentiates responses as a result of an increase in channel-opening probability.⁷

Because of the new evidence that anesthetics can enhance the effect of acetylcholine on the *Torpedo* nicotinic receptor, anesthesiologists and others interested in the molecular mechanisms by which anesthetics act will want to have answers to two questions (at least). The first is whether the same site is involved in the observed enhancement of acetylcholine binding and in channel block. The second is what are the properties of the sites (*e.g.*, how many and what affinity)? The paper by Raines and Zachariah¹ does not directly address the question of whether the change in apparent dissociation constant for desensitization could result from channel block. We cannot assume that the effects are independent; for example, if desensitization occurred at the same rate from both the open and the open-blocked states, the addition of isoflurane would increase the rate of desensitization at a particular concentration of acetylcholine by drawing more receptors into the open-blocked state. However, previous analyses of membrane currents by Dilger *et al.*^{8,9} provided evidence that potentiation by isoflurane is mediated by binding to a site other than the blocking site. Similarly, as already mentioned, studies of ethanol block and potentiation suggest that the actions are affected differently by specific point

mutations in muscle nicotinic receptors. What might the properties of this site be for isoflurane? The authors find that the EC₅₀ for the rate of desensitization by acetylcholine increases steadily with isoflurane concentration between 0.25 and 1.5%, with no sign of reaching a plateau. They argue that this suggests that there may be a number of sites of differing affinity. Underlying this suggestion is the assumption that occupation of each site results in a small additive change in gating efficacy. However, there could be a single class of site, in which occupancy is relatively far from saturated, even at the highest concentration tested. The idea of a multiplicity of sites is an interesting suggestion, which has not been thoroughly tested. The usual studies of potentiation involve measuring the steady state peak response of a receptor to a particular concentration of transmitter, which will show saturation as a function of potentiator concentration, regardless of the properties of the potentiator binding sites. Determination of the apparent transmitter EC₅₀ at a variety of potentiator concentrations (analogous to the current study) has been performed only rarely, although one study of pentobarbital potentiation of responses evoked by GABA shows some evidence of saturation in the GABA EC₅₀ as the pentobarbital concentration is increased from 10 to 100 μM .¹⁰

The work by Banks and Pearce² compares the effects of two volatile anesthetics (isoflurane and enflurane) on the amplitude and duration of miniature inhibitory postsynaptic currents (mediated by activation of GABA_A receptors) in hippocampal neurons studied in brain slices. Two effects are seen: the amplitude of the currents is reduced, and the duration is increased. Although both anesthetics produce both effects, the concentration dependencies are distinct, differing both between anesthetics and with one anesthetic for the two actions. The most parsimonious explanation is that the blocking and prolonging effects are mediated at different sites. This interpretation supports the general idea that the site involved in at least one form of enhancement of response differs from that involved in one form of block.

The results obtained with nicotinic receptors open the possibility that the technical armamentarium assembled to study the muscle nicotinic receptor can be brought to bear on the identification of sites involved in potentiation of responses of this receptor. Unfortunately, isoflurane cannot be used as a photoaffinity labeling compound, but other anesthetics may be used.¹¹ The use of point mutants has provided information on regions of the nicotinic receptor involved in inhibition by isoflurane⁶ and might be extended to address regions involved in potentiation. The results of these studies will surely guide analogous research of other

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members of the ligand-gated ion channel family, for which there is accumulating evidence for the existence of distinct sites, both for different classes of anesthetic and for the different actions of anesthetics.

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Goals- and Values-directed Approach to Informed Consent in the "DNR" Patient Presenting for Surgery

More Demanding of the Anesthesiologist?

This Editorial View accompanies the following article: Truog RD, Waisel DB, Burns JP: DNR in the OR: A goal-directed approach. *ANESTHESIOLOGY* 1999; 90:289-95.

On October 21, 1998, the American Society of Anesthesiologists' House of Delegates approved a revision of the Ethical Guidelines for the Anesthesia Care of Patients with Do-Not-Resuscitate Orders or Other Directives That Limit Treatment that added the third option of a goals- and values-directed approach. This revised document appears in the ASA Standards, Guidelines and Statements, dated October 1998. The full text for this appears on the ASA web site at the following address: www.asahq.org/standards

PASSAGE of the Patient Self-Determination Act in 1990 represented a response to public outcry against lingering authoritarianism and paternalism in the medical profession, a legacy of premodern medicine in which "arrogance"¹ was both an integral element of the art of medicine and an expression of adherence to the bioethical principle of beneficence. This federal legislation recognized the emergence of respect for patient autonomy as the predominating principle in biomedical ethics, and it was specifically designed to ensure patient self-determination for decisions about life-sustaining therapy. In this issue of the Journal, Truog *et al.*² recommend to anesthesiologists a practical approach for preserving the autonomy of the surgical patient with an existing do-not-resuscitate (DNR) order.

Autonomy refers to the patient's right to choose and

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