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Supersensitive Sites in the Central Nervous System

Anesthetics Block Brain Nicotinic Receptors

TWO papers appear in this issue of ANESTHESIOLOGY, reporting that some types of neuronal nicotinic receptors[1,2] are selectively inhibited by clinically used anesthetics. This new information goes beyond the level of "yet another anesthetic action" for two reasons. The first is that the actions occur on membrane proteins of the cellular target for anesthetics, the neuron. The second is that the actions of volatile anesthetics on neuronal nicotinic receptors are manifest at concentrations at the low end of the clinically relevant range (*i.e.*, substantially below MAC).

Neuronal nicotinic receptors are a family of acetylcholine-gated, cation-selective ion channels consisting of various combinations of homologous subunits ($\alpha 2-\alpha 9$, $\beta 2-\beta 4$) combined in a pentameric structure.[3,4] The most prevalent type of nicotinic receptor in the mammalian brain is composed of the $\alpha 4\beta 2$ subunit combination. The neuronal nicotinic receptor subunits are structurally related to those of several other transmitter-gated channels; these include the $GABA_A$ receptors, which have long been postulated to be important targets for anesthetic agents. Hence, it is not a complete surprise that anesthetics can act at these receptors. However, anesthetics produce markedly different effects on neuronal nicotinic receptors than they do on $GABA_A$ receptors; at nicotinic receptors, the anesthetics *inhibit* acetylcholine-mediated channel activation, whereas at $GABA_A$ receptors, anesthetic agents *potentiate* the agonist activity of GABA and can even act as agonists in their own right.

There are a number of similarities between the two studies reported in this journal issue. Both groups concentrate on receptors formed from $\alpha_4\beta_2$ subunits and examine the effects of volatile anesthetics (isoflurane, halothane, sevoflurane) and the intravenous anesthetic propofol. Both articles report that the concentrations volatile anesthetics producing half-maximal inhibition of responses to acetylcholine are lower than the concentrations required to produce surgical anesthesia; in contrast, propofol inhibits acetylcholine-induced currents of concentrations significantly higher than those required to produce surgical anesthesia. Both groups also report that some other nicotinic receptors are much less sensitive to anesthetics than those composed of $\alpha_4\beta_2$ subunits. Flood *et al.* tested the $\alpha 7$ neuronal nicotinic receptor (which in some respects resembles the muscle receptor most closely), whereas Violet *et al.* tested the muscle nicotinic receptor; both were found to be more than a log order less sensitive to the anesthetics than the $\alpha_4\beta_2$ receptors. Collectively, these data suggest the existence of selective, relatively high affinity binding sites for volatile anesthetics on $\alpha_4\beta_2$ neuronal nicotinic receptors.

Both papers also report observations that allow some inference to be made concerning the site with which anesthetics interact on the $\alpha_4\beta_2$ receptors. In the paper by Flood *et al.*, they find that neither propofol nor isoflurane is able to completely inhibit responses to acetylcholine. In the work reported by Violet *et al.*, they find that halothane is equally potent at blocking responses to low and high concentrations of acetylcholine. Neither of these observations can be reconciled with a simple competitive interaction between anesthetics and acetylcholine. Similarly, both groups make observations that indicate that an "open channel block" mechanism is unlikely. For simple open channel block, it would be expected that the inhibition should be more potent at a higher concentration of acetylcholine, which neither group finds. Hence, it seems likely that the interaction between these anesthetics (volatile anesthetics and propofol) and the neuronal nicotinic receptor occurs at some site other than the sites that bind acetylcholine or the channel lining itself. This is markedly different than the case of the structurally related muscle nico-

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tinic receptor, wherein the actions of volatile anesthetics and alcohols resemble classical open channel block and are associated with the portion of the receptor that lines the ion channel.[5–7]

There also are some significant differences between the two papers. First, Flood *et al.* used subunits cloned from the chicken, whereas Violet *et al.* used subunits from the rat. This difference may be of some consequence because there have been reports of significant differences between receptors reconstituted using subunits from the two sources. For example, the $\alpha_4\beta_2$ combination is activated at much lower concentrations of acetylcholine when the subunits are derived from chicken[8] (EC_{50} about 0.5 μM) than from rat[2] (EC_{50} about 100 μM). The overall similarity in observations suggests, however, that anesthetic inhibition overrides these quantitative differences. As mentioned previously, Flood *et al.* report that propofol and isoflurane produce only a partial block of response even at maximally effective concentrations. This observation may reflect some heterogeneity in receptors — for example, variable subunit stoichiometry or activation of muscarinic responses (as atropine was not used) — but rules out simple competition with acetylcholine and also simple channel block as mechanisms. Violet *et al.*, in contrast, see complete block of response at the maximal dosages of halothane, isoflurane, sevoflurane, or propofol. In addition, Flood *et al.* report that propofol and isoflurane are less potent at blocking responses to higher concentrations of acetylcholine (from their data, it is not clear whether the maximal block also is decreased at higher concentrations of acetylcholine). In contrast, Violet *et al.* find that a concentration of halothane that blocks responses to 160 μM acetylcholine by 50% is equally effective at blocking responses to 1 μM and 1600 μM acetylcholine. These discrepancies may indicate fundamental differences in the actions of anesthetics on the chick- and rat-derived receptors. However, they could result from an allosteric blocking model that was identical in both cases, but in which the efficacy of acetylcholine at gating or the efficacy of anesthetics at inducing the blocked state differed (for more discussion of such a model, see reference 9).

Finally one should ask, does anesthetic inhibition of neuronal nicotinic receptors have anything to do with producing anesthesia? This is a difficult question to answer, in part, because the role of neuronal nicotinic receptors in the functioning of the central nervous system is unclear. Nicotinic receptors do not have any major identified function as postsynaptic receptors — for example, they do not underlie excitatory transmission between neurons. For a number of years, therefore, it has been speculated that they may be located on presynaptic nerve terminals and serve to modulate the release of other neurotransmitters.[10,11] In most instances, activation of nicotinic receptors increases the release of transmitters and so inhibition of their function may result in a decrease in synaptic efficacy. Of course, the consequences of such a postulated decrease would depend on the particular pathways affected and the degree of depression. Unfortunately, few cognitive or behavioral consequences of nicotinic receptor function in the brain have been identified. This may be because their role is relatively diffuse, possibly in terms of memory, alertness, or ability to concentrate.

The second difficulty in addressing the potential role of neuronal nicotinic receptor inhibition in the anesthetic state is the observation that the receptors are maximally inhibited at volatile anesthetic concentrations that do not produce anesthesia. On the surface, this would suggest that neuronal nicotinic receptor inhibition should be irrelevant to anesthesia. However, it is important to remember that anesthesia comprises several distinguishable components, including amnesia, analgesia, and sedation, and that each of these effects may be produced by different concentrations of anesthetics. For example, it has been shown that memory is suppressed at concentrations of isoflurane much lower than those required to inhibit responses to noxious stimuli.[12] It is just possible that neuronal nicotinic receptors (or other unidentified targets affected by low concentrations of anesthetics) are responsible for producing particular components of the anesthetic state.

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References

1. Flood P, Ramirez-Latorre J, Role L: $\alpha4\beta2$ Neuronal nicotinic acetylcholine receptors in the central nervous system are potently inhibited by both isoflurane and propofol: $\alpha7$ Type nAChRs are unaffected. ANESTHESIOLOGY 1997; 86:859–65

2. Violet JM, Downie DL, Nakisa RC, Lieb WR, Franks NP: Differential sensitivities of mammalian neuronal and muscle nicotinic ace-

tylcholine receptors to general anesthetics. ANESTHESIOLOGY 1997; 86:866-74

3. Galzi JL, Changeux JP: Neuronal nicotinic receptors: Molecular organization and regulations. Neuropharmacology 1995; 34:563-82

4. McGehee DS, Role LW: Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu Rev Physiol 1995; 57:521-46

5. Wachtel RE, Wegrzynowicz ES: Kinetics of nicotinic acetylcholine ion channels in the presence of intravenous anaesthetics and induction agents. Br J Pharmacol 1992; 106:623-7

6. Dilger JP, Brett RS, Mody, HI: The effects of isoflurane on acetylcholine receptor channels: 2. Currents elicited by rapid perfusion of acetylcholine. Mol Pharmacol 1993; 44:1056-63

7. Forman SA, Miller KW, Yellen G: A discrete site for general anesthetics on a postsynaptic receptor. Mol Pharmacol 1995; 48:574-81

8. Bertrand D, Ballivet M, Rungger D: Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in Xenopus oocytes. Proc Natl Acad Sci U S A 1990; 87:1993-7

9. Ueno S, Bracamontes J, Zorumski CF, Weiss D, Steinbach JH: Bicuculline and gabazine are allosteric inhibitors of channel opening of the GABA-A receptor. J Neurosci 1997; 17:625-34

10. Steinbach JH: Mechanism of action of the nicotinic acetylcholine receptor, The Biology of Nicotine Dependence. Ciba Foundation Symposium, vol. 152. Edited by Bock G, Marsh J. New York, John Wiley & Sons, 1990, pp 53-67

11. McGehee DS, Role LW: Presynaptic ionotropic receptors. Curr Opin Neurobiol 1996; 6:342-9

12. Dwyer R, Bennett HL, Eger EI II, Heilbron D: Effects of isoflurane and nitrous oxide in subanesthetic concentrations on memory and responsiveness in volunteers. ANESTHESIOLOGY 1992; 77:888-98