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Anesthetic Effects on γ -Aminobutyric Acid A Receptors:

Not Just on Your Nerves

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In this current issue of *Anesthesiology*, Xiang *et al.*¹ describe the effects of volatile anesthetic activation of type A γ -amino butyric acid (GABA_A) receptors expressed on airway epithelial cells. It has long been appreciated that a key mechanism in the action of general anesthetics in the brain involves the anesthetics' allosteric potentiation of GABA on GABA_A receptors, thereby augmenting this inhibitory neurotransmitter system. However, the demonstration that GABA_A receptors are expressed extra-neuronally by a wide variety of cell types and modulate other important physiologic processes is a recent revelation. Although the bronchodilatory effects of volatile anesthetics have been clinically appreciated for many years, the signaling mechanisms by which this occurs in the lung are not fully known. The current study raises our awareness that anesthetics, *via* activation of GABA_A receptors, may be having many effects on multiple cell types in the lung that not only mediate bronchodilation, but also effect immunomodulation and mucus production by epithelial cells. The emerging discovery of GABA_A receptors on many peripheral cells raises important questions regarding the unrecognized effects of anesthetics on peripheral cellular processes.

The current study follows the seminal discovery (by these same authors) that multiple components of a GABAergic signaling system (GABA_A receptors and the enzyme that synthesizes GABA [glutamic acid decarboxylase]) are expressed in airway epithelium and play an important role in the modulation of mucus production.² In the current study, the authors focus on the alveolar type II epithelial (ATII) cells as they are also known to participate in immunomodulatory pulmonary processes (including specialized surfactant production and cytokine elaboration) which have important implications in inflammatory diseases of the lung such as asthma. With regard to the ATII cells, Xiang *et al.* advance our understanding of the GABAergic system in these cells by demonstrating the expression of multiple typical and atypical protein subunits of GABA_A receptors collectively from human cultured alveolar epithelial type II (A549) cells as well as *in situ* human peripheral lung sections. Additionally, they elegantly demonstrate on these human cultured ATII cells classical currents in response to exogenous GABA with positive allosteric modulation by isoflurane and sevoflurane. Most interestingly, isoflurane impairs the basal expression of a key proinflammatory enzyme, cyclooxygenase-2, presumably *via* decreased calcium entry following GABA_A receptor-mediated membrane depolarization. Opening of GABA_A channels in ATII cells favors chloride efflux and depolarization in contrast to mature neurons where opening of GABA_A channels favors chloride influx and membrane hyperpolarization due to differing electrochemical gradients in these cell types. These findings suggest a possible mechanism of anesthetics' antiinflammatory effects not only in

airway epithelial cells, but also on a host of other nonneuronal cells (including T lymphocytes) that have recently been found to express GABA_A receptors.

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It should be noted that the phenotype of GABA_A subunits that Xiang *et al.* report on human ATII cells is consistent with subunit expression observed when GABA_A receptors are found outside the neuronal synaptic cleft (*i.e.*, “extrasynaptic GABA_A receptors”). These extrasynaptic GABA_A receptors within the central nervous system seem to be limited to only a small or restricted number of subunits that include $\alpha 1, \alpha 4, \alpha 6$ (in association with Δ), or $\alpha 5$ (associating with either $\gamma 2$ or Δ).³ What is interesting is that in many nonneuronal tissues where GABA_A receptors are found, this extrasynaptic phenotype and specialized subunit combination seem to be maintained. Xiang *et al.*’s current study shows in human cultured A549 cells that $\alpha 5$ and Δ are present—which not only correlates well with the extrasynaptic phenotype, but also may explain the bidirectional electrophysiological effects of isoflurane that this group observed. These findings mirror the well-established observations that extrasynaptic GABA_A receptors are responsive to lower concentrations of GABA and display slower desensitization rates when compared to GABA_A receptors composed of subunits conforming to the more classical synaptic phenotype.⁴ Similarly, although unconventional, Xiang *et al.*’s observation that bicuculline could not completely reverse GABA-induced current is plausible, given published findings that shows competitive antagonists of the GABA binding site (such as bicuculline or gabazine) can exhibit differential blockade depending on GABA_A receptor subunit composition.^{5,6} Furthermore, differential blockade of GABA_A receptors is less established for the nonreversible antagonist picrotoxin, which agrees with Xiang *et al.*’s findings that this treatment abolished GABA-mediated current. This observation underscores an important point—that the role of the more “exotic” subunits (like the π subunit expressed in human ATII cells) may be modulating channel kinetics and pharmacological properties. This “theme” should be familiar to most anesthesiologists as pharmacologic specificity of benzodiazepine activation of GABA_A receptors requires the inclusion of a γ subunit in the receptor. As such, current medicinal chemistry efforts are directed at designing ligands with selectivity for different α subunits of GABA_A receptors that may allow selective targeting of specific neuronal or peripheral cell functions. However, caution is needed regarding potential subunit targeting of GABA_A receptors in epithelial cells given the lack of potential correlation Xiang *et al.* observed between human and mouse ATII cells in lung sections and human A549 cells in culture. They demonstrate the GABA_A $\alpha 2$ subunit in ATII cells in mouse lung sections but

the GABA_A α 5 subunit in cultured human A549 cells. If there is indeed a lack of complete concordance of GABA_A subunit expression in A549 cells between mice and human, continuing this work in another animal model where interspecies conservation is maintained, or (as was the case in this current article) working with human tissues and cells is essential to maintaining clinical relevance.

Perhaps the most intriguing component of this article is the potential link between GABA_A receptors and inflammation. Xiang *et al.* demonstrate GABA_A receptor-mediated reductions in the proinflammatory enzyme cyclooxygenase-2 in A549 cells treated with muscimol (a direct agonist of GABA_A receptors) and isoflurane (allosteric agonist), and the reversal of this effect under conditions where isoflurane and bicuculline were coadministered. We applaud the authors for this seminal discovery, as it could be paradigmshifting and establish another role for anesthetics beyond the central nervous system. However, while they certainly show reductions in constitutive expression of cyclooxygenase-2 (a key enzyme in arachidonic acid metabolism and prostanoid synthesis), it must be noted that the hallmark of this enzyme is that it is inducible and therefore exerts many of its proinflammatory cascades following an activating stimulus (such as lipopolysaccharide treatment).⁷ Although examining the effect of volatile anesthetics in attenuating cyclooxygenase-2 induction was beyond the scope of the current study, hopefully future research will address this important consideration.

Nevertheless, while one target of general anesthetics is GABA_A receptors in the central nervous system, a host of other cell types may have altered signaling events due to simultaneous activation of their GABA_A receptors with resulting changes in membrane potential and ion conductances. This may be particularly relevant in research related to the antiinflammatory effects and perhaps tumor surveillance aspects of leukocytes, a topic under intense clinical investigation with regard to cancer recurrence following exposure to opioids or local anesthetics. Perhaps the effects of activation of GABA_A receptors on tumor surveillance cells by intravenous and inhaled anesthetics are an equally important area in need of further research.

The current study's demonstration of an antiinflammatory role of isoflurane on airway epithelial cells follows a series of studies demonstrating a possible complex autocrine/paracrine signaling paradigm in the airway. Not only have GABA_A receptors and the enzyme that synthesizes GABA, glutamic acid decarboxylase, been described in airway epithelium and airway smooth muscle,⁸ but GABA transporters type 2 and 4 have been shown to release GABA from airway epithelial cells⁹ and metabotropic (Gi-coupled) type-B GABA receptors have been described on airway epithelium, smooth muscle, and nerves. Thus, the role of GABA on airway epithelial and smooth muscle function and its possible role in airway pathology (*e.g.*, asthma) are just beginning to be understood. Beyond the airway, the physiological effects of anesthetics on GABA_A channels expressed on peripheral cell types (*e.g.*, lymphocytes, renal tubule cells, and vascular and uterine smooth muscle) are largely unexplored and offer an opportunity for a new understanding of anesthetic effects on peripheral cells and organ function.

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References

1. Xiang Y-Y, Chen X, Li J, Wang S, Faclier G, MacDonald JF, Hogg JC, Orser BA, Lu W-Y. Isoflurane regulates atypical type-A gamma-aminobutyric acid receptors in alveolar type II epithelial cells. *Anesthesiology*. 2013; 118:1065–75. [PubMed: 23485993]
2. Xiang YY, Wang S, Liu M, Hirota JA, Li J, Ju W, Fan Y, Kelly MM, Ye B, Orser B, O'Byrne PM, Inman MD, Yang X, Lu WY. A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nat Med*. 2007; 13:862–7. [PubMed: 17589520]
3. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron*. 2012; 73:23–34. [PubMed: 22243744]
4. Glykys J, Mody I. Activation of GABAA receptors: Views from outside the synaptic cleft. *Neuron*. 2007; 56:763–70. [PubMed: 18054854]
5. Ueno S, Bracamontes J, Zorumski C, Weiss DS, Steinbach JH. Bicuculline and gabazine are allosteric inhibitors of channel opening of the gABAA receptor. *J Neurosci*. 1997; 17:625–34. [PubMed: 8987785]
6. McCartney MR, Deeb TZ, Henderson TN, Hales TG. Tonically active GABAA receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. *Mol Pharmacol*. 2007; 71:539–48. [PubMed: 17090706]
7. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol*. 1998; 38:97–120. [PubMed: 9597150]
8. Mizuta K, Xu D, Pan Y, Comas G, Sonett JR, Zhang Y, Panettieri RA Jr, Yang J, Emala CW Sr. GABAA receptors are expressed and facilitate relaxation in airway smooth muscle. *Am J Physiol lung Cell Mol Physiol*. 2008; 294:11206–16. [PubMed: 18408071]
9. Zaidi S, Gallos G, Yim PD, Xu D, Sonett JR, Panettieri RA Jr, Gerthoffer W, Emala CW. Functional expression of γ -amino butyric acid transporter 2 in human and guinea pig airway epithelium and smooth muscle. *Am J Respir Cell Mol Biol*. 2011; 45:332–9. [PubMed: 21057105]