Anesthesiology 2004; 100:469

Special Issue on Preconditioning: Work Presented at the October 2003 Journal Symposium

FOR the past 12 years, ANESTHESIOLOGY has organized and sponsored a Symposium at the Annual Meeting of the American Society of Anesthesiologists, based on a topic of contemporary interest identified by our Editorial Board. Each Symposium includes 10–20 poster/abstracts specially selected by the organizing Editors from among those submitted to the Annual Meeting. We also invite a number of speakers to review their work in the selected field and to participate in the discussions of the posters.

Both the Editorial Board and I have long believed that these sessions represent some of the best science that our specialty has to offer and certainly involve some of the best work presented at each year's meeting. Repeatedly, interested parties have said, "You should publish this material in the Journal," and this year we've decided to do just that. The October 2003 Symposium, entitled *Preconditioning against Ischemia and Reperfusion Injury*, was organized and moderated by Zeljko J. Bosjnak, Ph.D., and David C. Warltier, M.D., Ph.D., of the Medical College of Wisconsin, Milwaukee, Wisconsin. Speakers included David C. Warltier, M.D., Ph.D., Professor of Anesthesiology, Pharmacology and Medicine, Medical College of Wisconsin; Garrett J. Gross, Ph.D., Professor of Pharmacology & Toxicology, Medical College of Wisconsin; Stefan De Hert, M.D., Professor of Anesthesiology, University Hospital, Antwerp, Belgium; and Michael Zaugg, M.D., Head, Cardiovascular Anesthesia Laboratory, Institute of Anesthesiology, University of Zurich, Switzerland.

There were over 20 excellent posters. Before the meeting, all of the authors whose work was selected for the Symposium were asked to submit a formal manuscript describing their work. These manuscripts then underwent a full, but expedited, peer review process. The end result are the 15 articles that appear in the Special Section of this month's Journal—a series that truly represents some of the most sophisticated and up-to-date work being done currently in the field of preconditioning. Most (but not all) of the articles are derived from laboratory studies involving both the heart and brain, but my bet is that most anesthesiologists will have no difficulties grasping the implications of the work. To help in that process, the issue also contains a Review Article coauthored by Dr. David Warltier, updating our current understanding of the role of anesthesia and anesthetics on preconditioning in the heart.

The Journal's Web site (http://www.anesthesiology.org) offers the Symposium information in another format. All of the invited speakers agreed to being recorded, and they provided us with their slides. The result is four PowerPoint presentations with audio tracks of the same material presented to the actual Symposium attendees.

We hope that this will be only the first in a series of annual "special issues" derived from the Symposium. The topic for 2004, "*Pharmacogenomics and Anesthesia: Determinants of Individual Response and Outcome*," is being organized by Drs. Evan Kharasch (University of Washington, Seattle, Washington) and Margaret Wood (Columbia, University, New York, New York). Pharmacogenomics is the application of genomic concepts and technologies to the study of drug action, drug targets, pharmacokinetics, and therapeutic response. Pharmacogenomics is a subject of intense interest. Understanding the genetic factors responsible for interindividual variability in drug response and drug toxicity promises a future in which drug selection and dosing may become individualized. If you are an author working in this area, please make sure to submit your work to the Annual Meeting, and remember to check the box on the submission form indicating that you would like your work considered for the Symposium. We hope to see your paper here next March.

Additional material related to this editorial can be found on the ANESTHESIOLOGY Web site. Go to http://www.anesthesiology.org, click on Enhancements Index, and then scroll down to find the appropriate editorial and link. Supplementary material can also be accessed on the Web by clicking on the "ArticlePlus" link either in the Table of Contents or in the HTML version of the editorial. This Editorial View accompanies the Symposium articles in this issue.

Accepted for publication December 15, 2003. The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this editorial.

Michael M. Todd, M.D. Editor-in-Chief, ANESTHESIOLOGY, Department of Anesthesia, The University of Iowa, Iowa City, Iowa. anesthesiology@uiowa.edu

Anesthesiology 2004; 100:470–2

Anesthetic Effects on Glutamatergic Neurotransmission: Lessons Learned from a Large Synapse

SINCE the often-cited articles by Sowton and Sherrington,[1] Brooks and Eccles,[2] Bremer and Bonnet,[3] and Larrabee and Pasternak,[4] spanning nearly 50 yr of research into the cellular mechanisms of anesthesia, it has been almost axiomatic that depression of synaptic transmission by general anesthetics was not secondary to effects on the action potential. In this issue of the Journal, Wu *et al.*[5] apply cutting-edge electrophysiologic methods and a unique preparation from the mammalian brain to revisit this issue, and their results challenge this long-held and common conception—at least at a specialized synapse, the calyx of Held, and possibly at other excitatory glutamatergic synapses as well.

In the central nervous system (CNS), our understanding of the presynaptic effects of anesthetic drugs lags far behind our detailed knowledge of postsynaptic drug-receptor interactions. This mirrors the general delay in understanding the presynaptic machinery compared to the postsynaptic side of a prototypical CNS synapse.[6] The principal reason for this discrepancy lies in the technical difficulty of studying such small structures as axon terminals using electrophysiologic methods. A technical breakthrough came in 1994, when Forsythe[7] (followed within months by Borst *et al.*[8]) described a preparation in which both presynaptic and postsynaptic elements of a mammalian CNS synapse were accessible to direct electrophysiologic investigation. Since then, this preparation has contributed significantly to our understanding of presynaptic physiology in general, and to the release of glutamate, the most common excitatory neurotransmitter, in particular. Wu *et al.* have now used this preparation to address an important question in the field of anesthetic mechanisms.

The calyx of Held is a sign-inverting switch in the brainstem auditory pathway. Located in the medial nucleus of the trapezoid body, it plays a role in determining the spatial location of a sound source based on interaural intensity differences. This unique structure is specialized to provide reliable and rapid excitatory transmission from glutamate-releasing cells that originate in the anterior ventral cochlear nucleus onto the glycinergic neurons of the medial nucleus of the trapezoid body (note the switch from excitatory to inhibitory transmitter in the pathway). By CNS standards, it is a giant presynaptic terminal of 10–15 μM in diameter, and this makes it accessible to electrophysiologic recording using glass micropipettes. Each principal neuron in the medial nucleus of the trapezoid body receives input from only one calyx-type axon terminal, which, in a 9-day-old rat, comprises about 600 active release zones. Simultaneous recordings from the calyx presynaptic terminal and the postsynaptic neuron permit an unprecedented level of access to both partners of this excitatory CNS synapse.

The notion that the excitatory presynaptic terminal is a likely target for the depressant action of volatile anesthetics on neurotransmission has been suggested previously, based on electrophysiologic studies in the hippocampal slice preparation[9–11] and on biochemical studies of isolated cortical nerve terminals (synaptosomes).[12,13] However, the link between action potential invasion of the presynaptic terminal and response of the postsynaptic neuron involves multiple processes, including sodium channels, calcium channels, intracellular calcium stores, and a variety of proteins involved in vesicle docking and membrane fusion.[6,14] These and other presynaptic mechanisms have all been considered possible targets for volatile anesthetic action. Several observations, including the finding that some sodium channels may be more sensitive to depression than was previously appreciated, led to the suggestion that effects on voltage-gated sodium channels may play a role in reducing transmitter release,[15,16] but the relative importance of this and other targets remains unresolved. Wu *et al.* have now obtained direct measurements of the relative effects of isoflurane on two central events leading to the release of neurotransmitter: the presynaptic action potential and the fusion of transmitter-filled vesicles with the presynaptic membrane. The effect of isoflurane on the link between these two events—the increase in intracellular Ca^{2+}—was not measured directly but instead was extrapolated by simulating action potentials of varying amplitude in the presynaptic terminal and by measuring the resulting Ca^{2+} currents. Simultaneously, the postsynaptic responses to the released transmitter were also measured.

Wu *et al.* start by showing that anesthetic effects at the calyx of Held are qualitatively and quantitatively similar to those obtained in the more standard preparations (*e.g.*, the hippocampal slice) having synapses that are considered more representative of those in the CNS. The concentration of isoflurane that reduces by 50% the

This Editorial View accompanies the following article: Wu X-S, Sun J-Y, Evers AS, Crowder M, Wu L-G: Isoflurane inhibits transmitter release and the presynaptic action potential. ANESTHESIOLOGY 2004; 100:663–70.

Accepted for publication December 22, 2003.The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

postsynaptic response at the calyx (0.49 mM) is comparable to the concentrations of other volatile anesthetics that impair excitatory synapses in the hippocampus,[9] as is the observation that paired-pulse depression is reduced.[10,11] Having demonstrated that volatile anesthetic modulation of glutamate release at the calyx of Held is not unlike that of other CNS synapses, the authors provide new details of anesthetic interactions with the presynaptic terminal. Biologic membranes with electrochemical gradients (all excitable membranes) can "store" some electrical charge (capacitance). The fusion of transmitter-containing vesicles with the membrane of the presynaptic terminal leads to the incorporation of tiny amounts of new membrane into the terminal that can be measured as minute increases in the membrane capacitance of the presynaptic terminal. Because Wu *et al.* recorded directly from the presynaptic terminal, they were able to demonstrate that isoflurane reduced the capacitance change in response to presynaptic stimulation, indicating that isoflurane reduced the number of glutamate-containing vesicles that fused with the presynaptic membrane to release transmitter. Postsynaptic recordings demonstrated that the depressant effect of isoflurane on the capacitance increase quantitatively matched its depression of the postsynaptic response (43% *vs.* 50% at 0.7 mM isoflurane), supporting a presynaptic locus of action for its depressant effects on glutamatergic transmission.

Having thus demonstrated that isoflurane depresses transmitter release, Wu *et al.* addressed possible causes by studying the effects of isoflurane on two processes intimately linked to transmitter release: action potential and subsequent calcium entry. When an action potential traveling from the soma of the neuron invades the presynaptic terminal, Na^+ entry through voltage-activated channels leads to the initial depolarization of the terminal. Once depolarization reaches a certain threshold, various classes of voltage-gated Ca^{2+} channels open and Ca^{2+} enters, initiating the transmitter release process. The authors found that isoflurane depressed the action potential invading the presynaptic terminal only modestly (5.5% at 0.7 mM). Because of a nonlinear relationship, however, this modest effect on Na^+ entry translates into a substantial reduction of Ca^{2+} entering the terminal (approximately 12%). Transmitter release is in turn nonlinearly related to Ca^{2+} influx (the cooperativity ranges from 3–4 at various synapses). Therefore, in a short amplification cascade, a mere 5.5% depression of action potential amplitude translated into an approximately 50% reduction in the amount of transmitter released. Not all of the reduction in transmitter release caused by isoflurane could be accounted for by this effect, but a large fraction could—approximately 70%. The remaining 30% depression remains unresolved but might relate to direct effects on voltage-gated Ca^{2+} channels, or on the biochemical machinery that uses Ca^{2+} to allow transmitter-containing vesicles to fuse with the plasma membrane.

Wu *et al.* are not the first investigators to study the interaction of volatile anesthetics with axonal action potential propagation in the mammalian CNS. The general consensus has been that the effect of various anesthetics on presynaptic Na^+ channels was insignificant in myelinated axons.[17–19] The discrepancy between previous findings and the observations of Wu *et al.* must be reconciled. One possibility is that Na^+ channels expressed in the axon differ from those expressed in the terminal in their susceptibility to anesthetic block. Another possibility is that the susceptibility of the calyx demonstrated here is a developmental peculiarity: the shape of the action potential changes dramatically within days as the animals reach the age at which hearing begins (10–12 days in rats[20]). Wu *et al.* conducted their experiments using tissue from animals younger than 10 days old. However, similarities between anesthetic effects on overall function at these synapses and more mature synapses suggest that their results may be generally applicable. Last, it is certainly possible that extracellular recording techniques used in previous studies were not sensitive enough to consistently resolve such small changes in the action potential amplitude.

Wu *et al.* present strong evidence that isoflurane depresses glutamatergic synaptic transmission at relevant concentrations by reducing the amplitude of the action potential in the nerve terminal. Although it resolves some issues, this work, like all discovery, also leads to new questions. For example, do other volatile anesthetics act similarly? Does this result apply to all excitatory transmitters in the CNS? Is inhibitory transmission similarly depressed at the presynaptic level? The amplitude of evoked inhibitory responses is indeed decreased by isoflurane, but this has been attributed to direct anesthetic effects on postsynaptic inhibitory receptors.[21] There is also evidence that halothane can augment transmitter release.[22,23] What is different about these synapses, or about the action of this anesthetic? Finally, does depression of glutamatergic neurotransmission contribute to any endpoint of the multifaceted anesthetic state? In this context, it is interesting to recall that hypernatremia increases the minimum alveolar concentration of volatile anesthetics required to suppress movement in response to a noxious stimulus (MAC).[24] If glutamate release is so exquisitely sensitive to changes in the amplitude of the action potential, then elevation of $[Na^+]$ from 130 to 180 mM could, simply by increasing the driving force, more than account for the 75% increase in MAC—does this underlie the effect of sodium concentration on MAC?

Approximately one century ago, the calyx first described by Hans Held played a significant role in the debate between the supporters of the reticular hypothesis of the organization of the CNS and the proponents

of the "individual neuron." Held's observations led Ramon y Cajal and others to conclude that the CNS is made up of individual neurons. In the past decade, work on this synapse has helped to clarify numerous issues relating to the mechanisms of transmitter release, especially the contribution of various voltage-gated channels to this process. It is now helping us to understand the molecular mechanisms by which volatile anesthetics produce their long-studied, but heretofore poorly understood, effects.

Misha Perouansky, M.D.,* Hugh C. Hemmings, M.D., Ph.D.,† and Robert A. Pearce, M.D., Ph.D.* * University of Wisconsin, Madison, Wisconsin. † Weill Medical College of Cornell University, New York, New York. rapearce@wisc. edu

References

1. Sowton S, Sherrington C: On the relative effects of chloroform upon the heart and other muscular organs. BMJ 1905; 2:181–2
2. Brooks C, Eccles JC: A study of the effects of anaesthesia and asphyxia on the monosynaptic pathway through the spinal cord. J Neurophysiol 1947; 10: 349–60
3. Bremer F, Bonnet V: Action particuliere des barbituriques sur la transmission synaptique centrale. Arch Int Physiol 1948; 56:100–2
4. Larrabee MG, Posternak JM: Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. J Neurophysiol 1952; 15:91–114
5. Wu X-S, Sun J-Y, Evers AS, Crowder M, Wu L-G: Isoflurane inhibits transmitter release and the presynaptic action potential. ANESTHESIOLOGY 2004; 100: 663–70
6. Sudhof TC: The synaptic vesicle cycle revisited. Neuron 2000; 28:317–20
7. Forsythe ID: Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, in vitro. J Physiol 1994; 479:381–7
8. Borst JG, Helmchen F, Sakmann B: Pre- and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. J Physiol 1995; 489:825–40
9. Perouansky M, Baranov D, Salman M, Yaari Y: Effects of halothane on glutamate receptor-mediated excitatory postsynaptic currents: A patch-clamp study in adult mouse hippocampal slices. ANESTHESIOLOGY 1995; 83:109–19
10. MacIver MB, Mikulec AA, Amagasu SM, Monroe FA: Volatile anesthetics depress glutamate transmission *via* presynaptic actions. ANESTHESIOLOGY 1996; 85:823–34
11. Kirson ED, Yaari Y, Perouansky M: Presynaptic and postsynaptic actions of halothane at glutamatergic synapses in the mouse hippocampus. Br J Pharmacol 1998; 124:1607–14
12. Schlame M, Hemmings HC Jr: Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. ANESTHESIOLOGY 1995; 82:1406–16
13. Miao N, Frazer MJ, Lynch C3: Volatile anesthetics depress CA^{2+} transients and glutamate release in isolated cerebral synaptosomes. ANESTHESIOLOGY 1995; 83:593–603
14. Neher E: Vesicle pools and Ca2+ microdomains: New tools for understanding their roles in neurotransmitter release. Neuron 1998; 20:389–99
15. Rehberg B, Xiao YH, Duch DS: Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. ANESTHESIOLOGY 1996; 84:1223–33
16. Lingamaneni R, Birch ML, Hemmings HC: Widespread inhibition of sodium channel-dependent glutamate release from isolated nerve terminals by isoflurane and propofol. ANESTHESIOLOGY 2001; 95:1460–6
17. Richards CD, White AE: The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. J Physiol (Lond) 1975; 252:241–57
18. Berg-Johnsen J, Langmoen IA: The effect of isoflurane on unmyelinated and myelinated fibres in the rat brain. Acta Physiol Scand 1986; 127:87–93
19. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14
20. von Gersdorff H, Borst JGG: Short-term plasticity at the calyx of Held. Nature Reviews Neuroscience 2002; 3:53–64
21. Banks MI, Pearce RA: Dual actions of volatile anesthetics on GABA(A) IPSCs: Dissociation of blocking and prolonging effects. ANESTHESIOLOGY 1999; 90:120–34
22. Nishikawa K, MacIver MB: Membrane and synaptic actions of halothane on rat hippocampal pyramidal neurons and inhibitory interneurons. J Neurosci 2000; 20:5915–23
23. Westphalen RI, Hemmings HC: Selective depression by general anesthetics of glutamate versus GABA release from isolated cortical nerve terminals. J Pharmacol Exp Ther 2003; 304:1188–96
24. Tanifuji Y, Eger EI: Brain sodium, potassium, and osmolality: Effects on anesthetic requirement. Anesth Analg 1978; 57:404–10

Anesthesiology 2004; 100:472–3

One Thing Leads to Another

MS. Smith comes to clinic and reports having diffuse lower abdominal pain ever since her hysterectomy. When she voids, her bladder hurts. When she evacuates, her bowels hurt. She has aching in her back and legs. The gynecologists, urologists, and gastroenterologists have no answers. Her pain seems visceral in nature with poor localization and extreme sensitivity to activities of her internal organs, but no visceral disease is identified. What is going on? The basic science article by Shin and Eisenach[1] in this issue of the Journal suggests a potential cause for such a visceral pain: nerve injury. It has been long accepted that nerve injury can lead to back pain, leg pain, skin pain, almost any pain, but for some reason a link with visceral pain has not been commonplace. This article forms such a link. It demonstrates that peripheral nerve injury can result in both cutaneous and visceral hypersensitivity (a.k.a. possible pain states). At the same time, it demonstrates that the pharmacology of cutaneous hypersensitivity may not necessarily be extrapolated to visceral hypersensitivity. These observations are important for both intellectual and pragmatic reasons.

Intellectually, these results allow a sense of unity in the cognitive realm where similarities and dissimilarities must somehow be integrated. The authors' results suggest underlying principles associated with nerve dysfunction that are similar for all nociceptors. As a consequence, we do not have to invoke unique "protective" processes associated with one subtype of pain, and we must recognize the potential negative consequences of nerve injury in all sensory modalities. At the same time, neurochemical differences in the modulation of differing inputs seem to exist, which allows us to explain why all pain is not perceived as the same.

Now for the pragmatic issues. If we accept that peripheral nerve injury may result in visceral hypersensitivity, then we must widen our differential diagnosis regarding visceral pain complaints *and* we must consider the potential consequences of visceral nerve neurolysis as a therapeutic intervention. The first of these, a wider differential diagnosis, is not really a new consideration: nerve injury has long been proposed as a source of bowel and bladder dysfunction (outflow effects) and

This Editorial View accompanies the following article: Shin S-W, Eisenach JC: Peripheral nerve injury sensitizes the response to visceral distension, but not its inhibition by the antidepressant milnacipran. ANESTHESIOLOGY 2004; 100:671–5.

Accepted for publication October 16, 2003. The author is supported by grants from the National Institutes of Health, Bethesda, Maryland (No. NIDDK 51413 and No. NIDDK 66027). The author has no conflict of interest related to publication of this Editorial View or the related article.

more recently proposed as an etiology of neurogenic pelvic pain.[2] Others have gone so far as to propose that the painful bladder syndrome, interstitial cystitis, is a neuropathic, visceral, complex regional pain syndrome.[3] The more ominous issue is whether the practice of visceral nerve neurolysis for noncancer-related pain may create iatrogenic problems while trying to solve others. I have had a patient utter the frightening words "phantom pancreas" to me after a surgical splanchnectomy for chronic pancreatitis and at the time, I felt justified in discounting the idea as the whining of a chronic pain patient. Now I may need to reconsider.

Before agonizing over the harm I may have done, I will remember that this article is a first, basic science report. It relates to partial nerve injury—not total neurolysis—and there are other explanations for some of the data. Nerve-injured rats were compared with unoperated controls, so the deep-tissue–non-neural effects of the surgery could have contributed to effects on visceral sensitivity. Likewise, the cutaneous and visceral pharmacologic data cannot be directly compared, because one set of measures uses a threshold stimulus of ascending intensity and the other uses a fixed suprathreshold stimulus. Those interpretative issues aside, this information demonstrates an important relation between neural injury and the possibility of clinical pain. As more information becomes available, this may result in altered clinical practice.

Timothy J. Ness, M.D., Ph.D. Simon Gelman Professor of Anesthesiology, University of Alabama at Birmingham, Birmingham, Alabama. tim.ness@ccc.uab.edu

References

1. Shin S-W, Eisenach JC: Peripheral nerve injury sensitizes the response to visceral distension, but not its inhibition by the antidepressant milnacipran. ANESTHESIOLOGY 2004; 100:671–5

2. Zermann DH, Ishigooka M, Doggweiler R, Schmidt RA: Postoperative chronic pain and bladder dysfunction: Windup and neuronal plasticity—do we need a more neurological approach in pelvic surgery? J Urol 1998; 160:102–5

3. Galloway NT, Gabale DR, Irwin PP: Interstitial cystitis or reflex sympathetic dystrophy of the bladder? Semin Urol 1991; 9:148–53