

## EDITORIAL VIEWS

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### ***Snails, Spiders, and Stereospecificity—Is There a Role for Calcium Channels in Anesthetic Mechanisms?***

Depression of  $\text{Ca}^{2+}$  currents by volatile anesthetics has been described for more than a decade, having initially been noted in myocardial tissue<sup>1-4</sup> and subsequently in neural and secretory cells.<sup>5-8</sup> Yet, the minimal anesthetic contributions provided by the classic calcium channel blockers (dihydropyridines, phenylalkylamines, benzothiazepines) have not made a strong case for a calcium channel role in anesthetic mechanisms. However, molecular biologic techniques used to isolate genes encoding the major  $\text{Ca}^{2+}$  channel subunits combined with electrophysiologic investigations have distinguished at least five types of  $\text{Ca}^{2+}$  channels.<sup>9-11</sup> It is now clear that the aforementioned Ca channel blockers inhibit almost exclusively the L-type channel prominent in heart and vascular tissue but do not contribute to synaptic stimulus-secretion coupling.<sup>12</sup>

In this issue of ANESTHESIOLOGY, Study demonstrates isoflurane-mediated inhibition of  $\text{Ca}^{2+}$  currents at a relevant clinical concentration in a central nervous system cell isolated from the hippocampus.<sup>13</sup> These results emphasize the complexity of such an investigation, with the observed  $\text{Ca}^{2+}$  current generated by ion flux through at least four different  $\text{Ca}^{2+}$  channel types: T, L, N, and an additional unidentified type. The T-type channel, which activates at lower (more negative) membrane voltages than other classes, contributes a modest  $\text{Ca}^{2+}$  current that participates in pacemaker activity in some cells<sup>14,15</sup> and secretion in others in a variety of cell types.<sup>16</sup> Anesthetics depress T-type channels in heart,<sup>4</sup> endocrine,<sup>6</sup> and peripheral neuronal cells,<sup>7</sup> although the significance of these observations is unclear. Consistent with earlier work, Study reports that the dihydropyridine-sensitive L-type channels are inhibited by isoflurane. However, whereas the initial definitions of ion channels were based on biophysical criteria such as the voltage threshold required to open (still employed to identify T-type channels), such criteria as rate of inactivation do not always permit sep-

aration of the various high-voltage-activated channels. More recent studies have employed a variety of biologically derived toxins to define the various Ca channels that contribute currents in neuronal and other cells. The N-type channel, first described in peripheral neurons in 1985,<sup>17</sup> has been found to be selectively depressed by  $\omega$ -conotoxin GVIA, a poison from the marine snail *Conus geographus*.<sup>18,19</sup> Perhaps of greatest significance in this investigation is the evidence for isoflurane depression of this N-type  $\text{Ca}^{2+}$  channel that has been found in a variety of neural and secretory cell lines and has a key role in stimulus-secretion coupling in certain cells.<sup>20,21</sup> Anesthetic inhibition of the N-type channel would be of considerable importance in disrupting neuronal interaction.

Whereas Study demonstrated an action on several types of  $\text{Ca}^{2+}$  channels at clinical concentrations of isoflurane, Hall *et al.* in this issue report only very modest depression of  $\text{Ca}^{2+}$  currents from cerebellar Purkinje cells,<sup>22</sup> a cell line in which the P-type channel was initially defined.<sup>23</sup> The P-type channel, which is specifically blocked by a poison ( $\omega$ -Aga-IVA) from the funnel web spider *Agelenopsis aperta*, also appears to have a prominent role presynaptically and may be of critical importance for stimulus-secretion coupling in certain cells.<sup>21,23,24</sup> Because the cerebellum is far less involved in mood, memory, and consciousness than is the hippocampus, the relevance of these findings to anesthetic mechanisms is not obvious. Furthermore, whereas this channel is prominent in Purkinje cells, an  $\omega$ -Aga-IVA-resistant current is evident in other cells of the cerebellum<sup>25</sup> that may be sensitive to anesthetics. The experiments of Study reveal the tantalizing observation that an isoflurane-sensitive current remains after elimination of T-, L-, and N-type channels.<sup>13</sup> Although one could speculate that the toxin-resistant channel might be the P-type, its inactivation time course during depolarization and high sensitivity to isoflurane make this less likely. Unfortunately, the identity of this channel as a P-type could not be eliminated because of the lack of  $\omega$ -Aga-IVA, which is only recently commercially available. There is increasing evidence that other  $\text{Ca}^{2+}$  channels exist, tentatively termed Q and R,<sup>26</sup> that are resistant to all of the aforementioned toxins but sen-

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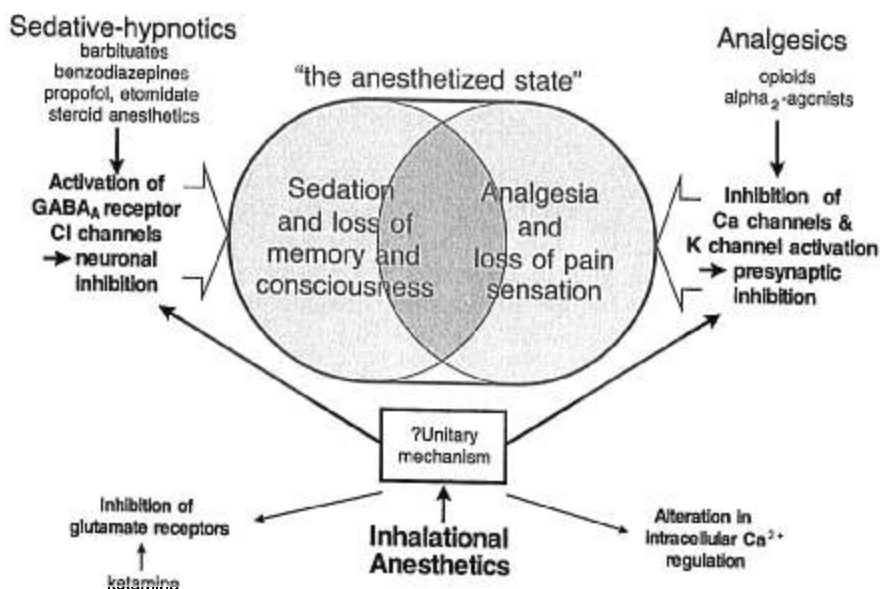
sitive to other *Conus* and *Agelenopsis* poisons (e.g., CVIIA, Aga IA). These channels also may play a prominent role in cell function. Molecular genetic studies confirm that genetic material for  $\text{Ca}^{2+}$  channels beyond T-, L-, N-, and P-types can be extracted from brain,<sup>11,27</sup> but the physiologic relevance and anesthetic sensitivity of these channels remain to be explored.

What do these observations regarding  $\text{Ca}^{2+}$  channels have to do with the mechanism of anesthetic action? Substantial evidence has accumulated, and was reviewed recently, describing the action of various agents that activate the  $\text{GABA}_A$  chloride channel.<sup>28</sup> Activation of this anion channel results in cell hyperpolarization or an increase in ion conductance that prevents depolarization, thereby inhibiting neuronal activity. Such activation by benzodiazepines, barbiturates, propofol, and the anesthetic steroids provides an obvious mechanism for the hypnotic effects of these agents.<sup>29,30</sup> Work by a number of investigators has shown that the volatile anesthetics have a similar action with regard to activating the  $\text{GABA}_A$  chloride channel<sup>31-34</sup> that provides a clear neurobiologic basis for the hypnotic and sedative effects of these anesthetics. Recently, the molecular interaction of anesthetics and the  $\text{GABA}_A$   $\text{Cl}^-$  channel has been probed by Harris *et al.* using the stereospecific isomers of isoflurane. They showed that isoflurane anesthesia, as assessed by sleep time in mice, appears to be stereoselective,<sup>35</sup> and that similar stereospecific volatile anesthetic actions can be demonstrated on  $\text{GABA}_A$  ligand binding.<sup>36</sup> Such stereospecific activation of a molluscan  $\text{K}^+$  current by the (+)-isomer of isoflurane at low concentrations (0.5 MAC) has been invoked as a strong argument for a direct anesthetic action on specific proteins and not a generalized bilayer effect,<sup>37</sup> even though not all anesthetics (e.g., chloroform, diethyl ether) possess a carbon atom with four different side chains and expressing stereoisomerism. Despite the fact that the phospholipids of the membrane bilayer are themselves all one stereoisomer of the glycerol backbone,<sup>38</sup> stereospecificity of action often is used as indicating a specific protein receptor as site of action. In this issue, Moody *et al.* provide evidence that isoflurane actions on L-type Ca channels are not stereoselective,<sup>39</sup> with supporting evidence by Graf *et al.*<sup>40</sup> based on studies in intact myocardium. Although it is tempting to attribute these nonstereoselective effects to a "nonspecific" (*i.e.*, lipid) site, the converse of the stereospecificity-protein receptor association is not necessarily true. In the absence of further evidence, the lack of a stereospecific effect does not necessarily

exclude a direct anesthetic effect on the channel protein. Interpretations of stereospecific actions of anesthetics require far greater investigation of small chiral compounds and how they interact with the various classes of biologic constituents, particularly the membrane phospholipids and membrane proteins.

Regardless of anesthetic action on the L-type  $\text{Ca}^{2+}$  channels, if anesthetic effects on the other classes of  $\text{Ca}^{2+}$  channels are similarly nonstereoselective, that seems to argue against  $\text{Ca}^{2+}$  channel effects mediating anesthesia, which appears to be somewhat stereoselective. Furthermore, a recent review<sup>41</sup> dismisses anesthetic effects on Ca channels as contributing to the anesthetic state based on the higher doses required to inhibit currents *in vitro*. Unfortunately, the authors neglected the higher anesthetic solubility at the temperatures of the *in vitro* experiments, and they failed to discuss in detail that synaptic release is a high-order function of the  $\text{Ca}^{2+}$  that enters during depolarization.<sup>42</sup> Thus, a reduction to 85% of control  $\text{I}_{\text{Ca}}$  may be amplified to a 39–50% depression ( $0.85^3$ ,  $0.85^4$ ) depending on the apparent cooperativity. Even if anesthetics alter Ca channel function, don't the effects at the  $\text{GABA}_A$  receptor that appear to be stereoselective<sup>36</sup> completely explain the clinical effects of the volatile anesthetics? The actions at the  $\text{GABA}_A$  receptor may account for the unconsciousness produced by the volatile anesthetics; however, an extrapolation beyond the hypnotic effect (sleep time) to an understanding of total anesthetic action seems unwarranted. Although the stereoselective correlation presented by Harris *et al.* between *in vivo*<sup>35</sup> and *in vitro*<sup>36</sup> results seems convincing, a few caveats must be presented. First, the stereoselectivity of the effect on sleep time was incomplete, with one isomer of isoflurane being only modestly more potent, especially at the higher concentrations. Second, complete study of anesthetic potency requires the use of noxious stimuli (such as tail-clamping in rats, or surgical incision, clinically). While Harris *et al.*<sup>35</sup> showed a stereoselective effect in terms of sleep time, it remains to be determined whether abolition of response to pain has a stereoselective component. Most importantly, it is evident to any practicing anesthesiologist that a complete anesthetic adequate for surgery cannot be provided by the  $\text{GABA}_A$ -agonist hypnotic agents alone, such as barbiturates, benzodiazepines, propofol, or etomidate. Likewise, while possessing considerable anesthetic potency, even the large doses of opioids and  $\alpha_2$ -adrenergic agents induce an incomplete anesthetic state that usually requires the addition of a hyp-

**Fig. 1.** The major pathways for sedative-hypnotics and analgesics in generating sedation and analgesia, respectively, which can combine to produce the anesthetic state. As indicated by the overlapping circles, the clinical effects of GABA<sub>A</sub> activation and neuronal Ca<sup>2+</sup> channel inhibition are unlikely to be exclusive. Activation of the same cellular processes by the volatile anesthetics may explain much of their anesthetic action, although the common "unitary mechanism" remains to be described. In addition to activation of GABA Cl<sup>-</sup> channels and inhibition of certain Ca<sup>2+</sup> channels, the volatile anesthetics may decrease Ca<sup>2+</sup> entry *via* excitatory glutamate receptors. Although the NMDA class seems particularly sensitive to ketamine, other glutamate-activated channels may be inhibited by the volatile agents. Volatile anesthetic effects also may be mediated in part by alteration of intracellular Ca<sup>2+</sup> stores.



notic agent for surgical anesthesia. Yet, a combination of a benzodiazepine, propofol, or barbiturate with an opioid or α<sub>2</sub>-adrenergic agent can produce an anesthetic state similar to that achieved by the volatile agents alone.

Most discussions of the "mechanism of anesthesia" are oriented toward volatile anesthetics and seek the elusive "unitary mechanism," neglecting that an equivalent anesthetic state can be generated by the aforementioned combined method. Since opioids and α<sub>2</sub>-agonists seem to "complete" the anesthetic when combined with GABA<sub>A</sub> activating agents, do the volatile anesthetics have cellular actions in common with the opioids, as they do with the GABA<sub>A</sub> activators? It has become clear that many of the opioids and α<sub>2</sub>-adrenergic agents act by inhibiting presynaptic Ca<sup>2+</sup> channels responsible for activating transmitter release.<sup>12,43,44</sup> The σ and μ opioid receptors, α<sub>2</sub> adrenergic receptors, GABA<sub>B</sub> receptors, and certain muscarinic receptors (present on sympathetic presynaptic regions) can decrease the amplitude of Ca<sup>2+</sup> currents that are carried by N-type Ca channels.<sup>44-49</sup> With regard to the paper by Study, we recognize that N-type as well as other Ca<sup>2+</sup> channels are depressed by isoflurane and probably by other anesthetics. In addition to effects on the Ca<sup>2+</sup> channels, activation of opioid and α<sub>2</sub> receptors increase the amplitude of presynaptic K<sup>+</sup> currents.<sup>50,51</sup> This hyperpolarizing action at the synaptic terminal also will reduce Ca<sup>2+</sup> entry and, consequently, decrease neurotransmitter release.<sup>52</sup> Investigations in the same cells

defining inhalational anesthetic and opioid actions, alone and in combination, will be critical to determine the possible common pathways of these agents whose relation to the anesthetic state then must be verified.

Although certainly an oversimplification of the complex neurophysiologic substrate, the major anesthetic mechanisms can be summarized in figure 1. Most prominently, the volatile anesthetics appear to bring about two important neurobiologic actions independently generated by: (1) the sedative-hypnotics, which activate GABA<sub>A</sub> channels, and (2) the opioids or α<sub>2</sub>-adrenergic agents, which reduce presynaptic Ca<sup>2+</sup> influx. Yet, at the molecular level, there remains the unanswered question of how the inhalational anesthetics can exert two apparently opposite channel effects: the GABA<sub>A</sub> Cl<sup>-</sup> channels stay open for a longer time and hyperpolarize the cell, whereas the Ca<sup>2+</sup> channels appear not to open or stay open for a shorter period. Is there a common pathway, *i.e.*, a "unitary mechanism"? Modest changes in the membrane lipids could influence these very different channel proteins in opposed directions. Better candidates for anesthetic sites of action include membrane-related cell processes that interact with and modulate ion channels. The G proteins provide receptor-effector coupling at the cytoplasmic membrane surface for myriad intercellular transmitters, including the opioids and α<sub>2</sub>-adrenergic agents. Another possible candidate is the protein kinase C enzyme family, whose mechanism of activation involves interaction with cell membrane lipids. This enzyme system, with

important modulating functions on both the GABA<sub>A</sub> receptors<sup>53</sup> and Ca<sup>2+</sup> channels,<sup>54,55</sup> was shown to be altered by anesthetic agents,<sup>56</sup> and protein kinase C inhibition lowers anesthetic requirements (at least in tadpoles).<sup>57</sup> Further work will be directed toward verifying these apparently distinct mechanisms and searching for a common cellular/molecular pathway by which volatile anesthetics can generate these effects on ion channel proteins and thereby inhibit cellular excitability.

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