Update on Neonatal Anesthetic Neurotoxicity

Insight into Molecular Mechanisms and Relevance to Humans

A FUNDAMENTAL premise of general anesthesia is that anesthetics produce a reversible state of unconsciousness and unresponsiveness. Implicit in this premise is that the brain and spinal cord are neurophysiologically the same before and after anesthesia. Recent experimental data have questioned the complete reversibility of anesthesia. In certain circumstances, anesthetic exposure in neonatal animals leads to neuronal death. Given the large number of neonates and infants who undergo surgery and anesthesia, the implications of these data for anesthesia in humans are readily apparent. Although the relevance of these findings to humans is a subject of heated debate, the unequivocal demonstration of neuronal death in animals exposed to clinically relevant concentrations of anesthetics has provoked significant concern among anesthesia care providers and patients. This editorial reviews new laboratory and clinical research advancing our understanding in this area that were presented at the Anesthesiology/Foundation for Anesthesia Education and Research Session at the Annual Meeting of the American Society of Anesthesiologists, Orlando, Florida, October 21, 2008.

The adverse impact from halothane exposure on the developing brain was reported two decades ago, when it was demonstrated that long-term exposure to halothane, beginning in utero and continuing for several days in the postnatal period, led to impaired synaptogenesis, reduced dendritic branching, suppressed axonal growth, and reduced myelination in rodents. Yet, these studies did not achieve notoriety because the manifestation of central nervous system toxicity required prolonged exposure to halothane, a situation not encountered in clinical practice.

Interest in anesthetic neurotoxicity was renewed by the demonstration that drugs that antagonize N-methyl-D-aspartate (NMDA) receptors and agonize γ-aminobutyric acid-A (GABA_A) receptors produce widespread neurodegeneration in the developing brain. These data led to a reevaluation of anesthetic neurotoxicity because commonly used anesthetic agents have these effects on NMDA and GABA_A receptors. In a seminal investigation, Jevtovic-Todorovic et al. demonstrated that exposure to isoflurane (0.75% to 1.5%) resulted in substantial neurodegeneration in a number of structures of the brain, including the hippocampus and neocortex. In addition, electrophysiologic function in the hippocampus was significantly reduced by anesthetics. Similar results were obtained by Fredriksson et al., who observed a reduction in cognitive function in rodents given a combination of thiopental or propofol and ketamine at postnatal day (PND) 10 at 8–10 weeks of age. These effects were not attributed to a disturbance in physiologic function (e.g., hypotension, hypoxia, or hypercarbia) because blood gas tensions have been reported to be normal. Moreover, Warner et al. evaluated anesthetic neurotoxicity in vitro in hippocampal slice preparations wherein blood flow is moot. In slices from 7-day-old pups, a 6-h exposure to isoflurane significantly increased neuronal death. In primate cortical neurons, Slikker et al. demonstrated that ketamine induced neurotoxicity; this group has also shown ketamine toxicity in neonatal monkeys in vivo. Anesthesia-induced injury has now also been demonstrated in the spinal cord. Collectively, these data indicate that anesthetic agents are toxic to the brain and that this injury results in a long-term impairment of cognitive function. Such neurotoxicity has now been demonstrated not only for isoflurane but also for ketamine, midazolam, diazepam, pentobarbital, thiopental, nitrous oxide, and propofol.

The mechanisms by which anesthetics produce neuronal death are under intense investigation. Many of the agents that produce neurodegeneration are antagonists of the NMDA receptor, which include ketamine and nitrous oxide. It is well established that glutamate signaling via the NMDA receptor plays a crucial role in synaptic development and neuronal survival. During the critical period of synaptogenesis, inhibition of NMDA receptor signaling is detrimental to brain development. Indeed, other NMDA antagonists also produce a pattern of neurodegeneration that is similar to that produced by ketamine. Volatile anesthetics, propofol, barbiturates, and benzodiazepines are GABA_A agonists, and each of these is associated with neuronal toxicity. In the adult, GABA_A receptor activation leads to an influx of Cl⁻ into the cell. This results in hyperpolarization and can lead to neuroprotection in many models of hypoxia and ischemia. However, in the developing brain, especially during synaptogenesis, intracellular concentration of Cl⁻ is high; activation of GABA_A receptor results in Cl⁻ efflux and depolarization of the neuron. Consequently, depolarization-mediated rise in intracellular calcium concentration reaches levels that can be harmful to the cell, suggesting that this excitotoxic action of GABA may contribute to neuronal injury. Although the precise mechanisms by which injury is produced are not clear, an imbalance between excitatory and inhibitory input in the central nervous system during synaptogenesis may...
trigger apoptosis, apoptosis occurs by both the intrinsic and extrinsic pathways. What remains to be clarified is how NMDA antagonism, GABA<sub>α</sub> agonism, and an imbalance between excitation and inhibition leads to the activation of apoptotic pathways.

This issue of Anesthesiology contains several investigations that have advanced our understanding of not only the molecular mechanisms that underlie anesthetic neurotoxicity in the developing brain, but also the means by which this injury can be mitigated. Moreover, two important studies address the relevance of anesthetic neurotoxicity in rodents to that in human infants and children.

The investigation of Straiko et al. sheds light on how ketamine and propofol sedation can lead to apoptosis. The administration of ketamine or propofol to PND5 mouse pups decreased phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2) and kinase (Akt) levels and led to neuroapoptosis. Lithium restored phosphorylated ERK1/2 levels (but not Akt) and prevented ketamine- and propofol-induced injury. The activation of NMDA receptors, either by direct application of NMDA or by increasing neuronal activity, results in the activation of a variety of signaling mechanisms that enhance neuronal survival; these mechanisms include activation of calmodulin-dependent protein kinase,<sup>20</sup> Akt<sup>21</sup> ERK1/2, release of brain-derived neurotrophic factor (BDNF),<sup>22</sup> and inhibition of glycogen synthase kinase 3β.<sup>23</sup> Suppression of pERK1/2 and pAkt by ketamine is not surprising given its potent antagonist effect at NMDA receptors. Propofol might also reduce NMDA receptor signaling by reducing neuronal activity. Therefore, the data of Straiko et al. indicate that ketamine and propofol trigger apoptosis by suppressing prosurvival signaling in developing neurons.<sup>19</sup> Of interest is the finding that lithium restored pERK1/2 but had no effect on pAkt. These data suggest that activation of several prosurvival pathways simultaneously may not be necessary for the prevention of apoptosis; activation of a single survival pathway may be sufficient. If so, then there is a rich variety of targets by which apoptosis might be prevented. These could possibly include calmodulin-dependent protein kinase, Akt, and antagonism of glycogen synthase kinase 3β. It should, however, be noted that activation of ERK1/2 was demonstrated only with the higher dose of lithium (6 mg · kg<sup>−1</sup>) that was studied. Although 3 mg/kg did inhibit apoptosis, its effect vis-à-vis ERK1/2 activation remains to be defined. In addition, lithium also prevented apoptosis that is a normal part of brain development. During brain development, the number of cells generated is substantially greater than what is required; the final number of neurons in the adult brain is a function of survival of specific neurons and elimination of excess neurons by apoptosis.<sup>25</sup> The potential impact of preventing the normal process of neuronal elimination in the developing brain is not clear.

It is now understood that anesthetics can cause neuronal apoptosis and that exposure during the critical period of synaptogenesis can lead to subsequent neurocognitive dysfunction. The conventional concept is that anesthesia-induced cell death is the cause of behavioral abnormalities. Stratman et al. have challenged this view of anesthetic neurotoxicity.<sup>26,27</sup> These investigators exposed rat neonatal pups to 1 minimum alveolar concentration of isoflurane anesthesia for 1 h, 2 h, or 4 h on PND7. A carbon dioxide control group was also studied, given that isoflurane caused a substantial respiratory depression and increased arterial carbon dioxide tensions. Injury was evaluated 12 h after exposure. In separate groups of pups, cognitive function was evaluated in fear conditioning and water maze paradigms 8 weeks after exposure. Widespread neuronal injury (hippocampus, cortex, thalamus) was apparent in pups exposed to isoflurane for 2 h and 4 h but not for 1 h. In addition, hypercarbia per se led to significant injury to the thalamus only. Of significant interest was the observation that neurocognitive dysfunction was apparent only in the 4-h isoflurane group; although injury was observed in the 2-h isoflurane and hypercarbia groups, cognitive deficits were not apparent. In a separate investigation, the same authors demonstrated a cognitive deficit in fear-conditioning and water maze paradigms 6 weeks after exposure but not at earlier (15 and 26 days after exposure).<sup>27</sup> On the basis of these findings, the authors posit that the behavioral deficits that were observed after neonatal isoflurane exposure could not be attributed entirely to cell death. What is the evidence that supports this conclusion? Although hypercarbia caused cell death and was not associated with a cognitive deficit, the injury was restricted to the thalamus, and no injury was observed in the hippocampus. Similarly, with 2-h isoflurane anesthesia, some injury was observed in the hippocampus but it was less than in the 4-h anesthesia group. It is possible that a threshold level of injury to the hippocampus must occur for a cognitive deficit to be manifest. It is therefore plausible that the extent of cell death in the hippocampus in the hypercarbia and 2-h isoflurane groups was not sufficient to result in a cognitive deficit. This therefore does not support the authors’ premise that factors other than cell death contribute to cognitive dysfunction. Their argument, however, is buttressed by their observation that behavioral deficits were not observed early after exposure (2–5 weeks) but were apparent 6 weeks later. If cognitive deficit is due primarily to cell death, a deficit should have been apparent at earlier time points. The fact that this was not seen suggests that other mechanisms must also be operative.

One mechanism by which neonatal isoflurane exposure might lead to sustained hippocampal-dependent cognitive deficits might be an adverse effect of isoflurane on hippocampal neurogenesis. Neurogenesis is critical to normal hippocampal function, and even limited sup-
pression of neurogenesis is associated with the development of significant cognitive deficits. There is now clear evidence that isoflurane exposure reduces the rate of neuronal progenitor cell proliferation in vitro and suppresses neurogenesis in vivo. This effect of isoflurane was apparent as late as 4 days after exposure. Importantly, unlike neurons in the cortex and caudate, anesthetics do not induce apoptosis in progenitor cells. Are anesthetic-induced cognitive abnormalities related to a transient suppression of neurogenesis? Although the data of Stratmann et al. do not allow for a firm conclusion to be drawn, it should be noted that a single dose of ethanol and low doses of ionizing radiation are both associated with a significant reduction and in hippocampal neurogenesis and subsequent hippocampal dependent cognitive deficits. Therefore, isoflurane suppression of neurogenesis as a cause for cognitive deficits is certainly within the realm of possibility. What remains to be clarified is the means by which isoflurane exerts its adverse effect. In the developing rodent brain, dentate granule cell neurogenesis begins approximately 1 week before birth and peaks at about PND7. Thereafter, the rate of neurogenesis decreases gradually and reaches adult levels within the next few weeks. The process of neurogenesis involves proliferation of neuronal precursor cells, differentiation into neurons and glia, migration and functional integration into the hippocampal circuitry (see Zhao et al. and Piatti et al. for review). In these new neurons, GABAA currents are excitatory and lead to depolarization; this is of importance in the maturation and integration of the neuron into the hippocampal circuitry. At about 3 weeks, GABAA currents become inhibitory and cause hyperpolarization. Glutamatergic inputs are established thereafter. Of note is the importance of NMDA receptor activity to the survival of the new neuron. Survival and integration of the new neuron is therefore influenced by neuronal activity in their environment. Given the potent activity of anesthetics at GABAA and NMDA receptors, it is possible that anesthetics might interfere, not only with proliferation of progenitors, but also their survival, maturation, and integration into the hippocampal circuitry. To what extent anesthetics interfere with the entire process of neurogenesis is not currently clear, and experimental clarification of anesthetic effect at each stage of neurogenesis is needed.

The impact of anesthetics on the developing brain is not limited to neuroapoptosis and neurogenesis. Work by Vutskits et al. indicates that anesthetic exposure fundamentally alters dendritic spine formation and therefore synaptic function. The majority of excitatory synapses are formed on dendritic spines. During the early postnatal period, motile and transient protrusions called filopodia emerge from dendritic shafts. These filopodia interact with other filopodia or with axons to form nascent synapses. With the progression of synapse development, filopodia are gradually replaced by dendritic spines. These spines are characterized on the basis of shape: thin, stubby, or mushroom spines. A considerable body of evidence suggests that the size of the spine head is directly correlated with the strength of the synapse; these spines are stable and are less plastic. Thin spines, on the other hand, preferentially undergo long-term depression. Spines, therefore, are of critical importance to synaptic transmission. When Vutskits et al. exposed mice (PND 15, 20, 30, and 90) to propofol, ketamine, or midazolam for 5 h, significant remodeling of spines in the apical dendrites of layer 5 pyramidal neurons of the somatosensory cortex was observed in PND15 and PND20 mice but not in the older mice. This remodeling consisted of an increase in dendritic spines and filopodia but a decrease in spine diameter; these changes persisted for 2 weeks after anesthesia exposure. Neuroapoptosis was not observed in any group. These findings led the authors to suggest that the administration of anesthetics during the brain growth spurt might interfere with the appropriate development of neural circuitry. This study raises several possibilities. First, dendritic spine modulation was not accompanied by neuroapoptosis. This indicates that the potential adverse impact of anesthetic exposure is not just limited to cell death but also to fundamental development of neuronal networks. Second, the alteration in spine development was apparent in pups at PND15 and PND20. This is well beyond the window of neuronal apoptosis (peak at PND7 and not apparent at PND 14). These data suggest that the window of vulnerability to anesthetic exposure is greater than previously thought and extends to at least PND20. Finally, neurogenesis is an ongoing process and continues throughout life. New neurons have to be integrated into neuronal networks. A provocative possibility is that anesthetics might interfere with the integration of new neurons at any stage of life, not just during the neonatal brain growth spurt. Undoubtedly, these possibilities will be explored in future studies.

The evidence from the preclinical studies presented in this issue and those from earlier studies by other investigators naturally begs the following question: Are these findings clinically relevant? Further, what is the available evidence from existing clinical studies that could directly address this question? In this issue of Anesthesiology, there are two clinical investigations that make the attempt to directly address the potential anesthetic effects on the developing brain in children. Before now, those clinical studies that have examined neurodevelopmental outcome in neonates and young infants after surgery and anesthesia have mostly involved specific populations of infants with serious comorbidity such as prematurity, low birth weight, or congenital cardiac defects. Not only were the effects of exposure to anesthetics on neurodevelopmental endpoints not specifically analyzed, the relative contribution of anesthetic exposure would probably be difficult to decipher given the significant comorbid
conditions found with these patient groups. There has been a body of literature on the effects of anesthetics on postoperative behavior in children, but the follow-up period in these studies has been limited to no more than 2 months.\textsuperscript{53--57} Therefore, they do not adequately address the potential long-term neurobehavioral outcome. In summary, the limitations of the data from available clinical studies include: (1) absence of direct examination of the anesthetic effects on neurodevelopmental outcome, (2) insufficient time for follow-up assessment of long-term outcome, and (3) lack of well defined endpoints in neurodevelopmental outcome.

The two studies in this issue of Anesthesiology specifically examine the effects of anesthetic exposure in infants after surgery and anesthesia, with the assessment of the effects performed long after the exposure of anesthetic has taken place. These studies thus addressed two of the three significant limitations of our existing literature outlined above. Both groups of authors used retrospective cohorts of infants who had surgery for their study, but the actual procedures, the age under examination and the outcome endpoints differed between the two studies. Wilder \textit{et al.}\textsuperscript{58} examined a wide variety of surgical procedures in infants under 3 yr of age, and Kalkman \textit{et al.}\textsuperscript{59} specifically focused on infants who had urological procedures before the age of 2 yr. The endpoints employed in the two studies were divergent. Wilder \textit{et al.} used reports of learning disability of any kind as the endpoint. The study findings were based on linking the data on learning disability in the Olmsted County birth cohort with the extensive medical and anesthetic records that the investigators were able to review. This made possible the detailed analysis of the specifics of anesthetic exposure, including the type of agents, the duration, and frequency of exposure. Kalkman \textit{et al.} used a validated questionnaire on child behavior to identify deviant behaviors as the endpoint for their study. In both of these studies, the findings may be considered preliminary but provocative. These studies provide suggestive evidence that anesthetic exposure at a young age may be associated with increased risk for later learning disability when there has been more than one exposure and "deviant" behavior. Therefore, the two studies used very different outcome measures to assess the effects of anesthesia. Using learning disability as an outcome measure may be clinically attractive, but it also has several important limitations. Learning disability may involve three different types, namely language, verbal, and math, which clearly have divergent neurobiological bases. Unlike validated neuropsychological instruments, there is no standardization in the assessment for learning disability. Finally, learning disability may be significantly influenced by genetic, family and socioeconomic factors. The outcome measure used by Kalkman \textit{et al.} was abnormal behavior based on the validated Child Behavior Check List questionnaire completed by the parents. There are several limitations in using the Child Behavior Check List for the stated study goal. First, the Child Behavior Check List does not provide any information directly on any domain of cognitive function other than behavior.\textsuperscript{60} Second, the Child Behavior Check List is only a useful screen for problematic behavior, and confirmation of deviant behavior through evaluation of psychopathology is recommended.\textsuperscript{61} Although both of these studies make important contributions in furthering our current state of knowledge with respect to the clinical relevance of anesthetic neurotoxicity in the developing brain, they also underscore the challenges in defining the appropriate endpoints or outcome measures.

The findings from the preclinical studies in this issue may be instructive in the search for the appropriate endpoints of neurodevelopmental outcome for evaluation. They indicate that the hippocampus, a region known to be functionally important in the mechanisms of memory and learning, may be the most vulnerable. Therefore, the most relevant neurodevelopmental outcome endpoint probably should include testing those cognitive functions linked to the hippocampus, specifically, a more detailed assessment in memory and learning. Coincidentally, evidence from previous animal studies also found that neonatal anesthetic exposure was associated with deficits in memory, attention, learning, and motor function in these same animals as adults. The downside of this strategy is that it would not evaluate those neurodevelopmental outcomes that may be uniquely human. Both executive function and verbal language development are examples of neurocognitive functions that gain greater prominence with maturation.

In summary, substantial progress into the mechanisms by which anesthetics injure the developing brain in rodents and the means by which this injury can be mitigated has been made. The available data clearly indicate that anesthetics lead to widespread neuroapoptosis when the exposure occurs during synaptogenesis, with peak vulnerability being at about 1 week postnatal age. There is a commonality in the processes that are involved in anesthetic induced abnormal apoptosis and normal physiologic apoptosis. This highlights the difficulty of developing therapeutic interventions to prevent toxicity. Inhibition of anesthetic-induced neuroapoptosis may also inhibit normal developmental apoptosis; the adverse impact of preservation of neurons otherwise destined to death will require experimental clarification. It should, however, be noted that anesthetic neurotoxicity is not limited to apoptosis. Transient suppression of neurogenesis and alteration in the development of neuronal circuitry also contribute to the total injury. With respect to the latter, the brain appears to be susceptible to anesthetic neurotoxicity up to 3 weeks after birth. This suggests that the duration of the period of vulnerability of the brain has to be reevaluated, and the window of vulnerability perhaps has to be extended.

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Ultimately, what still remains is the question of the relevance of anesthetic neurotoxicity in animal models to human pediatric anesthesia. Although two retrospective studies herein suggest that a correlation between anesthetic exposure early in life is associated with learning and behavioral abnormalities later in life, the data cannot be considered to be evidence of the existence of anesthetic neurotoxicity in humans. The absence of rigorously conducted prospective randomized trials precludes recommendations on clinical practice. Nevertheless, the findings from the two clinical studies presented in this issue highlight the urgent need for the conduct of one or more large-scale human studies, with well-defined outcome measures and an appropriate follow-up period that specifically examine the effects of anesthesia and surgery on cognitive development in pediatric patients. It is therefore encouraging that the United States Food and Drug Administration recently launched the first phase of the SAFEKIDS (Safety of Key Inhaled and Intravenous Drugs in Pediatrics) initiative to provide some seed funding for several clinical projects. The ultimate goal of this initiative is to construct a private-public partnership that would support clinical investigations that would provide sufficient data so that clinicians and parents can make informed decisions with respect to the safety of anesthesia in children.

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