Catherine E. Creeley, PhD

John W. Olney, MD

Millions of human fetuses, infants, and children are exposed to anesthetic drugs every year in the United States and throughout the world. Anesthesia administered during critical stages of neurodevelopment has been considered safe and without adverse long-term consequences. However, recent reports provide mounting evidence that exposure of the immature animal brain to anesthetics during the period of rapid synaptogenesis, also known as the brain growth spurt period, triggers widespread apoptotic neurodegeneration, inhibits neurogenesis, and causes significant long-term neurocognitive impairment. Herein, we summarize currently available evidence for anesthesia-induced pathological changes in the brain and associated long-term neurocognitive deficits and discuss promising strategies for protecting the developing brain from the potentially injurious effects of anesthetic drugs while allowing the beneficial actions of these drugs to be realized.

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to undergo natural cell death during the developmental period.8–10 However, evidence from studies using newly developed methods for identifying and counting apoptotic profiles documents that the bulk of natural cell death occurs in proliferating cell populations that have not yet differentiated into neurons.11–13 Thus, although 50% of neural and/or glial precursors may die during development, normally the vast majority of differentiated neurons successfully become integrated during the period of synaptogenesis and only a relatively small percentage undergo apoptosis. The original observation that developing neurons are obliged to commit suicide if they fail to make appropriate synaptic connections is accurate, but this observation was made in the context of experiments in which the process of synaptogenesis was intentionally being thwarted. During normal development, synaptogenesis is not being experimentally manipulated, but exposing developing neurons to anesthetic drugs is an unnatural event that can disrupt synaptogenesis and cause apoptotic death of many neurons that would have otherwise survived and made a positive contribution to the functions of the brain.

MECHANISMS UNDERLYING DRUG-INDUCED DEVELOPMENTAL NEUROAPOPTOSIS

Studies using Bax knockout mice and infant rats have revealed that the mechanism of cell death is Bax dependent and involves downregulation of bcl_x, mitochondrial injury, and extramitochondrial leakage of cytochrome c. This is followed by a sequence of changes culminating in activation of caspase-314–18 and also may involve brain-derived neurotrophic factor-dependent and death receptor-dependent pathways.17,18 Results of studies using caspase-3 knockout mice15 suggest that commitment to cell death occurs before the caspase-3 activation step, which signifies that immunohistochemical detection and quantification of neurons positive for activated caspase-3 (AC-3) provide a reliable means of mapping cells that have already progressed beyond the point of cell death commitment. Accordingly, AC-3 immunohistochemistry has been used extensively for marking dying neurons in recent studies focusing on drug-induced developmental neuroapoptosis.14–16,19–29

Mechanisms that mediate Bax translocation to mitochondrial membranes remain elusive, but mitogen-activated plasma kinase systems have been implicated in several studies, especially the extracellular signal-regulated protein kinase (ERK) pathway. Suppression of ERK phosphorylation seems to be a common property shared by numerous drugs that trigger developmental neuroapoptosis, including alcohol,30 several anesthetic drugs,31 and several AEDs.32 The potential role of intracellular signaling mechanisms in anesthesia-induced developmental neuroapoptosis is discussed further below in relation to methods for protecting against this phenomenon.

PERMANANCE AND FUNCTIONAL SIGNIFICANCE OF DRUG-INDUCED DEVELOPMENTAL NEUROAPOPTOSIS

Initially, it was questioned whether drug-induced neuroapoptosis causes permanent neuronal losses, or if permanent, whether they are likely to have any functional significance. We present evidence addressing this question for each class of apoptogenic drugs.

Ethanol

It has been demonstrated by counting missing neurons weeks or months after drug exposure that a high dose of ethanol permanently deletes up to 68% of the neurons in certain brain regions; evidence for replacement of the missing neurons by neoneurogenesis was looked for but not found.19,28 Wozniak et al.28 demonstrated that a one-time exposure of infant mice to ethanol causes widespread neurodegeneration and profound learning/memory deficits in adolescence followed by apparent partial functional recovery in adulthood, although appearances of partial recovery may have been attributable to repeated testing and acquired familiarity with the task. Klintsova et al.33 found that neonatal exposure to ethanol suppresses neurogenesis that normally occurs during adulthood in dentate hippocampal neurons and results in a permanent reduction in the number of dentate neurons. In a series of studies, it has been shown that ethanol triggers neuroapoptosis widely throughout many regions of the central nervous system (CNS), including the forebrain, midbrain, cerebellum, brainstem, spinal cord, and retina.2–4,19–21,34,35 Ethanol can have severe deleterious effects on the human fetal brain resulting in a syndrome (fetal alcohol spectrum disorder) featuring widespread pathomorphological changes in the brain, long-term learning disability, and other neuropsychiatric disturbances.

Antiepileptic Drugs

Bittigau et al.5 demonstrated that all of the AEDs used frequently in the treatment of pediatric epilepsy trigger neuroapoptosis in the developing rat brain at clinically relevant doses. Valproate caused neuroapoptosis at a relatively low dose and was severely neuroapoptogenic at higher doses. This is of interest in relation to pediatric medicine in that it was recently reported, based on a multicenter study,36 that children of mothers who were exposed to valproate for management of epilepsy in the third trimester of pregnancy have a 9-point deficit in intelligence quotient at the age of 3 yr. Stefovska et al.37 reported that AEDs suppress neurogenesis in the dentate hippocampus, as has also been reported for ethanol (see above) and anesthesia (see below). Ikonomidou et al.38 have shown in a human neuroimaging study that prenatal
exposure of the human fetus to AEDs causes a reduced mass of the striatum, which is a finding that has been reported repeatedly in neuroimaging studies of children exposed prenatally to ethanol.39

Anesthesia

Six years ago, Jevtovic-Todorovic et al.4 reported that exposure of infant rats to a clinically relevant cocktail of anesthetic drugs (midazolam, nitrous oxide, and isoflurane) for 6 h caused widespread neurodegeneration in the developing brain and neurocognitive deficits that persisted through adolescence into adulthood. Subsequently, several research groups have reported that subanesthetic exposure to individual anesthetic drugs, including ketamine,22 midazolam,22 propofol,23 isoflurane,24,25 sevoflurane,26 and chloral hydrate,27 trigger a significant neuroapoptosis response in the infant rodent brain. Fredriksson et al.40–42 exposed infant mice to the NMDA antagonist, ketamine, or GABA agonists (diazepam, thiopental, and propofol) and demonstrated that these drugs, especially if used in combination, can cause apoptotic neurodegeneration and long-term locomotor and cognitive deficits. Nitrous oxide, by itself at subanesthetic concentrations, reportedly triggers little or no neuroapoptosis, but it markedly augments isoflurane-induced neuroapoptosis in the infant rat brain. Satomoto et al.43 exposed infant mice to sevoflurane for 6 h and found evidence for widespread neuroapoptosis plus long-term learning disability and abnormal social behaviors. Stratmann et al.44 have reported widespread neuronal degeneration and permanent neurocognitive deficits in rats after exposure to isoflurane in infancy. These authors have also reported that isoflurane suppresses neurogenesis, which raises the question whether the neurocognitive deficits are linked to suppressed neurogenesis or to the widespread neuronal degeneration (or to both). Slikker et al.26 have reported that exposure of 5-day-old infant rhesus macaques to ketamine produces age- and dose-dependent neuroapoptosis in the cerebral cortex and long-term disturbances in learning and memory.46 In the initial report by this group, they exposed the infant macaques for 3 or 24 h and reported a significant neuroapoptosis response only after the 24-h exposure. However, in a more recent study,47 these authors found a significant neuroapoptosis response in infant rhesus macaques after a 9-h exposure to ketamine. Brambrink et al.48 exposed 6-day-old infant rhesus macaques to isoflurane for 5 h and observed a 13-fold increase in neuroapoptosis widely distributed across all divisions of the neocortex. In addition, there was a 10-fold increase in gliapoptosis distributed throughout the white matter regions in these isoflurane-exposed infant macaque brains.49 This finding, which has also been described in fetal macaque brains exposed to ethanol,50 may be of clinical significance in that the dying glia are in the oligodendroglial lineage, and they seem to be particularly vulnerable in the maturational stage when they are just beginning to myelinate axons that interconnect neurons throughout the CNS.

HUMAN RELEVANCE OF DRUG-INDUCED DEVELOPMENTAL CNS APOPTOSIS

It has been vigorously debated whether animal data pertaining to anesthesia-induced neuroapoptosis are relevant in a human clinical context.51–53 Unfortunately, the issue cannot be settled by debate or by appeal to the archival literature because there is a paucity of well-designed studies aimed at clarifying whether exposure of the immature human brain to anesthesia has adverse long-term consequences.54 A troublesome reality that has discouraged research in this area is the extreme difficulty in separating anesthesia as a variable from other relevant variables such as the impact of surgery and of comorbid factors associated with chronic illness.54–57 Despite this obstacle, epidemiological researchers are beginning to focus research attention on this issue, and data reported recently suggest that brief anesthesia exposure during early infancy may be associated with increased risk for learning disabilities.58–60 These findings are all of a preliminary nature and, as such, are not a suitable basis for definitive conclusions or decision making. Moreover, these data pertain primarily to undefined periods of brief anesthesia exposure during large blocks of time (e.g., first 4 yr after birth) and do not adequately address important issues such as whether infants 1 wk old are more vulnerable than children 4 yr old, whether prematurely born infants or in utero third trimester fetuses are more vulnerable than full-term neonates, whether prolonged anesthesia exposure for many hours poses greater risk than brief exposure for less than an hour, whether certain anesthetic drugs or drug combinations are more toxic than others, and so forth.

It is generally agreed that animal data pertaining to drug toxicity are of heuristic value for planning human studies, but animal studies cannot resolve the issue of human susceptibility. Human studies can perhaps resolve the issue, but not in a timely manner, and perhaps not at a high level of confidence. Even if multiple epidemiological studies demonstrate a strong association between anesthetic exposure and developmental disabilities, it will remain a debatable issue whether the disability should be attributed to anesthesia or to the impact of the surgical procedure, or concurrent illness or to a multitude of other confounding variables.54–57 Moreover, if it is finally determined by consensus opinion that anesthetic drugs do delete neurons from the developing human brain, what can be done to prevent this deleterious iatrogenic outcome? We have proposed that it may be feasible to develop methods that are safe and do not interfere with the beneficial properties of anesthetic drugs but...
can inactivate the intracellular signals that trigger neuroapoptosis. We have been exploring neuroprotective strategies and have identified 2 approaches that we consider particularly promising. One is a pharmacological approach and the other is a temperature regulation approach.

**Pharmacological—Lithium**

Lithium has long been used as a first-line maintenance therapy for bipolar disorder and has also been used off-label in the treatment of other psychiatric disorders. There is evidence from *in vitro* and *in vivo* animal studies suggesting that it may have neuroprotective properties relevant to adult neurological disorders such as Alzheimer’s disease and stroke, but it has never been proposed or approved for uses targeting the developing nervous system. Zhong et al. reported that lithium protects the infant mouse brain against neurodegeneration induced by ethanol. We have confirmed this finding and have presented evidence that the neuroprotective mechanism may involve a counterbalancing interaction between ethanol and lithium in which ethanol suppresses phosphorylation of ERK and lithium prevents ethanol from suppressing this phosphorylation process. ERK is a mitogen-activated protein kinase signaling molecule that has long been implicated in the regulation of cell survival, so the ERK signaling pathway is a logical candidate for complicity in cell death processes.

The above findings pertaining to an ethanol/lithium/ERK interaction prompted us to explore the possibility that anesthetic drugs might also suppress phosphorylation of ERK and that lithium might counteract this action and protect neurons against anesthesia-induced neuroapoptosis. We found that subanesthetic doses of ketamine and propofol do rapidly suppress ERK phosphorylation, and lithium prevents this action, while also completely eliminating the neuroapoptosis reaction induced by these anesthetic drugs. Remarkably, a single dose of lithium administered at the same time that the anesthetic drug is administered confers complete protection against the neuroapoptosis process and does not seem to interfere with the beneficial properties of anesthetic drugs. An additional and potentially problematic feature of lithium’s action is that it suppresses the natural apoptotic process by which redundant or faulty neurons are deleted from the developing brain. Whether lithium’s ability to suppress naturally occurring developmental neuroapoptosis has any lasting consequences remains to be determined.

Lithium’s toxicity potential for immature humans has been studied primarily in the context of lithium being administered chronically to pregnant women who have bipolar disorder. It has been described as a weak teratogen based on evidence in some studies (disputed in others) that chronic exposure of the developing fetus during the first trimester of pregnancy may be associated with an increase in cardiac anomalies. Chronic exposure during later stages of pregnancy is currently considered a safe practice.

Whether exposure of a third trimester fetus or young infant to a single dose of lithium has toxic consequences is unknown, because there has been very little research addressing this specific question. Clearly, before lithium could be recommended as a protective therapy against anesthesia-induced developmental neuroapoptosis, it would have to be evaluated much more thoroughly for both efficacy and safety.

It is worth emphasizing that even if lithium *per se* is found to be unsafe or unsuitable for use as a neuroprotector against anesthesia-induced neuroapoptosis, the observation that it does effectively counteract this neurotoxic process, apparently by a counterbalancing action in specific intracellular signaling pathways, provides realistic hope that a pharmacological means for controlling this neurotoxic process can be developed. It is also realistic to believe that further research aimed at understanding the intracellular mechanisms underlying lithium’s protective action will lead to the development of other drugs that might be superior to lithium for this therapeutic purpose.

**Temperature Regulation—Hypothermia**

Hypothermia can prevent neuronal degeneration induced in the developing brain by hypoxia/ischemia, and hypothermia is currently becoming recognized as a potentially effective treatment for perinatal encephalopathy. Hypoxia/ischemia triggers an early cell death response and a late cell death response in the developing rodent brain, and the early response is mediated by an excitotoxic mechanism, whereas the delayed response is mediated by an apoptotic mechanism. Hypothermia was originally shown to be protective against the excitotoxic component and has not been adequately studied by methods that would clearly demonstrate protection against the apoptotic component. Cell death induced by anesthetic drugs is strictly apoptotic, and it is thought that suppression of neuronal activity is instrumental in triggering this apoptotic process. This raises a very interesting question: What would happen if you suppress neuronal activity throughout the brain by head cooling and at the same time suppress neuronal activity by exposure to an anesthetic drug?

We undertook some pilot experiments to address the above question. Initially, we conducted experiments to determine how changes in ambient temperature (AT) influence brain temperature (BT) in 4-day-old (P4) ICR infant mice. We found that immediately after removing the pups from underneath their mother, their mean (+SEM) BT was 34.3°C ± 0.5°C (*n* = 6). We then removed pups from the nest and kept them for 5 h in a container partially submerged in a temperature-regulated water bath maintained at 1 of 3 ATs (35°C, 30°C, or 25°C). Hourly BT measurements (*n* = 6 pups per condition per hour) revealed that the mean BT (MBT) averaged across the 5-h period for infant mice
incubated under these 3 AT conditions was 34.7°C, 31.9°C, and 29.7°C, respectively.

To test the influence of BT on the neuroapoptotic action of anesthetic drugs, we elected to compare 2 temperature extremes, the MBT = 34.7°C condition versus the MBT = 29.7°C condition. We refer to these conditions as normothermic and hypothermic, respectively. We exposed some groups of P4 ICR mice \((n = 8\) per group) to isoflurane, ketamine, or no anesthesia at a normothermic MBT (34.7°C) and other groups to the same treatment conditions at a hypothermic MBT (29.7°C). Isoflurane was administered as a vaporized gas at 0.75% concentration (in 30% oxygen/remainder air) for 4 h, and ketamine was administered intraperitoneally at a dose of 40 mg/kg. All pups were killed 5 h after initiation of treatment for histological evaluation of their brains, using AC-3 immunohistochemical staining and a stereological approach that is well established \(^{2–5,22,23,25,28,30–32,48,49}\) for mapping and quantifying neurons undergoing apoptosis.

The notable findings were 3-fold: 1) Pups treated with either isoflurane or ketamine under the normothermic condition had a significantly increased density of AC-3–positive profiles compared with the normothermic controls not exposed to anesthesia. 2) The density of AC-3–positive profiles after treatment with these drugs under the hypothermic condition was equal to or less than in the brains of normothermic controls (no anesthesia), signifying that under the hypothermic condition, isoflurane and ketamine did not induce a neuroapoptosis response. 3) The density of AC-3–positive profiles in hypothermic control pups was significantly reduced to approximately half that in normothermic control pups, indicating that hypothermia suppressed the rate of spontaneous neuroapoptosis in control brains not exposed to anesthesia. These findings, together with statistical analyses, are presented in Figure 1.

In this study, a significant neuroapoptotic reaction was observed when the MBT was 34.7°C (comparable to the MBT when the pup is nestled under its mother). In previous studies \(^{22,23,25,30,31}\) we have demonstrated a significant neuroapoptotic response when the AT was 30°C, which corresponds to an MBT of 31.9°C (see above). Thus, a reduction in MBT of 2.8°C (from 34.7°C down to 31.9°C) does not significantly retard the neurotoxic process, but an additional 2.2°C reduction down to 29.7°C does prevent it from occurring, at least within the acute time frame of the present experiments.

Thus, we have tested the hypothesis that brain cooling might augment the neuroapoptosis response induced in the developing brain by anesthetic drugs, and the results clearly support the opposite conclusion: brain cooling to only a moderate degree markedly suppressed the neuroapoptosis response to either isoflurane or ketamine in the infant mouse brain. We conclude that brain cooling warrants further investigation as a potentially safe and effective means of preventing anesthetic drugs from injuring the developing brain. Before brain cooling could be recommended for this purpose, important questions beyond the scope of our pilot studies must be addressed. What is the optimal time, in relation to anesthesia exposure, for applying hypothermia, and how long should it be continued after anesthesia exposure? When hypothermia is applied in an optimal manner, does it provide permanent protection against anesthesia-induced developmental neuroapoptosis, or does it merely postpone the expression of this neurotoxic process?

**SUMMARY AND CONCLUSIONS**

Herein, we have reviewed evidence pertaining to the potential of anesthetic drugs to cause neuroapoptosis in the developing brain and subsequent neurocognitive impairment. At the time of this writing, the evidence for human susceptibility is suggestive but not definitive. If the evidence for human susceptibility becomes increasingly more compelling, it would be valuable to have a safe and effective means of preventing this neurotoxic phenomenon while not interfering with the beneficial actions of anesthetic drugs. In this article, we have described 2 neuroprotection strategies (lithium treatment and hypothermia) that we consider particularly promising. Each approach will require additional research to further clarify both safety and efficacy.
The observation that both lithium pretreatment and hypothermia suppress spontaneous developmental neuroapoptosis that occurs naturally in the developing brain raises an important, interesting question: Does transient suppression of spontaneous developmental neuroapoptosis have adverse neurodevelopmental consequences? This question is relevant to the potential use of hypothermia in pediatric medicine not only for protection against anesthesia-induced neuroapoptosis but also for preventing neuroapoptosis associated with other neuropathological conditions affecting the developing brain, including hypoxia/ischemia and traumatic brain injury.

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Preventing Anesthetic Neurotoxicity


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