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Between Clotho and Lachesis

How Isoflurane Seals Neuronal Fate

"Alle Ding sind Gift, und nichts ohn Gift; allein die Dosis macht, daß ein Ding kein Gift ist."
"All things are poison and nothing is without poison, only the dose permits something not to be poisonous."
— Theophrastus Phillippus Aureolus Bombastus von Hohenheim, known as Paracelsus (1493–1541)

FOR more than 150 yr, general anesthetics have represented the quintessential triumph of medical pharmacology, providing painless oblivion for patients and a quiet field for surgeons. But now, concerns over long-term sequelae in the vulnerable brain have begun to tarnish the heretofore almost spotless image of general anesthetics. The extremely low therapeutic ratios of these powerful agents (which would likely preclude regulatory approval today if not for their extreme utility) are well known; indeed their expert use and careful dosing forms the essence of the practice of anesthesiology. However, it is the reports of *delayed* and *long-term* damage to the developing and the aged brain that have attracted attention and concern from both scientists and the public.‡ The sophisticated and timely report by Head *et al.* in this issue of ANESTHESIOLOGY[1] illuminates some the complexity and context-specificity of anesthetic interactions with the mammalian brain.

Head *et al.* investigated the interactions of isoflurane with neuronal growth factor brain-derived neurotrophic factor (BDNF)[2] and tissue plasminogen activator (tPA), a serine protease better known for its hematologic role in fibrinolysis and its therapeutic application as a thrombolytic agent.[3] BDNF-dependent signaling had been implicated previously in the activation of neuroapoptosis by a triple anesthetic cocktail (nitrous oxide, midazolam, isoflurane) in neonatal rats *in vivo*.[4] Head *et al.* extend these findings by providing evidence that exposure to isoflurane alone reduces release of tPA from neurons and thereby upsets the delicate balance between survival-promoting and death-promoting aspects of BDNF signaling. This effect is proposed to be critical to isoflurane-mediated neurotoxicity in the developing rodent brain by reducing the processing of pro-BDNF to mature BDNF. This hypothesis connects the physiologic mechanisms that regulate neuronal survival with the neuropharmacology of anesthetics.

The developing brain is a veritable battleground between factors promoting neuronal survival and death. Many more neurons and synapses are formed throughout development than survive into adulthood to form the 10^{11} neurons and 10^{14} synapses in the adult human brain. Programmed cell death (apoptosis) and synaptic pruning are critical to plasticity and stabilization of circuits in the developing nervous system. These are active processes tightly controlled by complex neurotrophin signaling mechanisms to ensure normal development, facilitate synaptic plasticity, and prevent neoplasia. The four mature neurotrophins, which include BDNF, nerve growth factor (NGF), neurotrophin-3, and neurotrophin-4, activate one or more of the three Trk (tropomyosin-related kinase) transmembrane receptor tyrosine kinases. Dimerization of these receptors induced by neurotrophin binding activates an intrinsic receptor tyrosine kinase and subsequently downstream intracellular signaling pathways that ultimately control neuronal survival, differentiation, and connectivity.[5] Neuronal activity regulates secretion of BDNF, which influences synaptic efficacy and morphology *via* activation of presynaptic and postsynaptic TrkB receptors. Although BDNF is clearly critical to normal neuronal development and survival, it also has a dark side. BDNF can be secreted in its precursor form as pro-BDNF, which, far from being an inactive precursor, binds to and activates $p75^{NTR}$, an alternative pan-neurotrophin receptor that binds all neurotrophins with similar affinity.[6] This receptor is structurally unrelated to Trk receptors, but it is a member of the tumor necrosis factor family of receptors and contains a type II intracellular death domain. Activation of $p75^{NTR}$ by pro-BDNF initiates signaling pathways that can lead to trophic effects at low levels and to apoptosis with more intense stimulation.[7,8] Under normal conditions, BDNF and pro-BDNF maintain a fine balance between neuronal life and death that might be upset by anesthetics.[9]

Head *et al.* hypothesized that tPA might play the deciding role of the Fate Atropos of Greek mythology. In contrast to the shears of the most feared and powerful of the Moirae, however, the proteolytic shears of tPA enable survival rather than signal death. In addition to its well known role in intravascular fibrinolysis (conversion of plasminogen to plasmin), tPA can cleave pro-neurotrophins (including pro-BDNF) to their mature forms in the brain. By studying the effects of isoflurane exposure on markers for neuronal apoptosis and dendritic spines

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‡ Shekar C: Anesthesia: A medical mainstay re-examined. Available at: http://articles.latimes.com/2007/may/14/health/he-anesthesia14. Accessed December 30, 2008.

in cultures of rat hippocampal and cortical neurons, these investigators provide evidence that isoflurane reduces dendritic spine number and increases apoptotic neuronal death after 5 days, but not after 2 weeks, in culture (roughly equivalent to 4 and 6 months gestation in humans, respectively). This is supported by ultrastructural evidence *in vivo* that isoflurane exposure of 5-day-old rat pups reduces the number of synapses. These effects are attributed to reduced vesicular secretion of tPA, which the authors speculate is the result of depressed neuronal activity. This reduction in tPA release diminishes proteolytic processing of pro-BDNF to BDNF, shifting the balance of power from the survival-promoting spinner of life Clotho (BDNF/TrkB signaling) to the proapoptotic life-terminating Lachesis (pro-BDNF/$p75^{NTR}$ signaling).

This intriguing proposal is supported by complementary neurobiological evidence that implicates specific elements of critical signaling pathways in neuronal survival and spinogenesis. Overall, Head *et al.* provide a case against isoflurane that has interesting testable implications. Several controversial aspects of the case will require confirmation. For example, previous evidence that pro-BDNF is secreted and then processed extracellularly was obtained from cultured cells engineered to overexpress the growth factor[10] and so might not apply to physiologic conditions where pro-BDNF processing takes place intracellularly and therefore would not require activity-dependent release,[11] a key aspect of the current model. Demonstration of isoflurane-induced inhibition of *activity-dependent* release of BDNF/proBDNF and/or tPA is critical to solidify support for the proposed model. Previous work has shown that triple cocktail anesthesia can reduce or increase BDNF levels in cortex or thalamus, respectively[4]; therefore, regional differences in signaling mechanisms could result in region-specific anesthetic effects. Further insight into the affected types of synapses (excitatory *vs.* inhibitory, spine *vs.* shaft), histologic confirmation of the age-dependent and possibly cell- or region-dependent increase in neuroapoptosis, and clarification of the roles of matrix metalloproteinase-7, which can also cleave pro-BDNF to BDNF, will provide additional evidence to seal the case. The critical question of causation, *i.e.*, whether dendritic, synaptic, and neuronal loss represent durable changes that lead to neurobehavioral deficits in later life,[12] will not be answered by these cell biologic studies, but will require complementary neurobehavioral testing.

These data support the notion that isoflurane has deleterious effects on dendritic and synaptic stability and neuronal survival in neonatal rodents. Taking their findings a step further, Head *et al.* show that a cell-permeable peptide inhibitor of $p75^{NTR}$ signaling can prevent isoflurane-induced apoptosis and loss of synapses and filopodial dendritic spines in their model. Together with the protection provided by β-estradiol,[4] L-carnitine,[13] and lithium,[14] these findings provide leads for targeted therapeutic approaches for the protection of the vulnerable rodent brain. Nevertheless, a number of crucial hurdles remain to establish the clinical significance of experimental observations relating to the neurotoxicity of anesthetics. First, available evidence indicates that isoflurane exposure (concentration times duration) must be substantial to be detrimental, and must occur within a narrow developmental window. In the current example, toxicity was observed in neurons from rats equivalent to postnatal day 5 but was absent by postnatal day 14. This narrow window of vulnerability (the exact timing is region-specific) correlates with lower levels of BDNF secretion early in development that might contribute to this sensitivity.[15] This narrow window of susceptibility is consistent with studies of alcohol toxicity in rats[16] and ketamine toxicity in monkeys.[17] Translating such developmental windows between species is not trivial,[18] so the relevance of effects observed in altricial rodents for precocial humans is difficult to extrapolate.

Even more intriguing is the notion that the end-result of isoflurane-induced reduction of tPA release (and hence plasmin levels) might be determined by exposure time and context. Increased tPA/plasmin levels have been implicated in stroke, seizure, and other ischemia/neurotoxicity models,[19] and tPA contributes to increased permeability of the blood-brain barrier and hence to cerebral edema in the ischemic brain[20] such that under these pathologic conditions inhibition of tPA secretion might be beneficial. On the other hand, tPA and plasmin might also contribute to β-amyloid clearance in the Alzheimer Disease-susceptible brain,[21] another potentially important interaction between anesthetics and brain pathophysiology of unclear significance.

In addition to the "objective" context, the "subjective" and individualized context is gaining importance in the era of perioperative genetic screening. A common genetic polymorphism (Val66Met) in the BDNF gene results in reduced BDNF release and is associated with anxiety-related behavior in mice[10] and susceptibility to neuropsychiatric disorders and memory impairment in humans. Diminished expression of BDNF tilts the balance toward depression, and increased BDNF expression enhances responsiveness to antidepressant treatment.[22] Could the Val/Met BDNF polymorphism be associated with increased risk to isoflurane-induced neurotoxicity, and might antidepressant treatment be protective?

Five centuries later, an update to the maxim of Paracelsus is in order. It is not only the administered dose that distinguishes between a poison and a remedy, but also the timing and context of the dose.

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