

## Anesthesia and cerebral apoptosis

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**Abstract** : General anesthetics interact with targets at the cellular and molecular levels. They have the potential to induce changes in the body and the brain. Usually, these interactions are thought to be short lasting.

In contrast, recent evidences suggest that alcohol, a toxic sharing many mechanisms with general anesthetics, induces long term effect at these levels.

This is particularly evident in the period of synaptogenesis during which alcohol can induce excessive cerebral apoptosis (histopathologic changes) in juvenile animal models.

Even if the vast majority of our patients seems to completely restore homeostasis after general anesthesia, we don't know if the changes induced at the brain level in animal models exist in human.

This article intends to supply biological, pharmacological and experimental basis for a possible long term effect of general anesthetics on the human developing brain.

**Key words** : Anesthesia, general ; Anesthetics gases ; Anesthetics i.v. ; Brain, anesthesia, molecular effects ; Neonates.

### INTRODUCTION

Intrauterine exposure of the human fetus to ethanol causes a well studied dysmorphogenic and neuropathological syndrome called fetal alcohol effects (FAE) or fetal alcohol syndrome (FAS) (1). At the brain level, ethanol induces pathological changes. These are characterized by pathological apoptosis and excessive brain mass loss.

As these effects of ethanol on the brain are thought to be mediated by its action on GABA (gamma-aminobutyric acid) and NMDA (N-methyl-D-aspartate) receptors, which are also the main targets of most general anesthetics, these agents might be suspected to have the same effects as ethanol on the human developing brain by inducing excessive and pathological cerebral apoptosis.

To understand this possible phenomenon, we have to explain what is apoptosis and its mechanisms.

### APOPTOSIS

Apoptosis is the programmed cell death (or cellular suicide) in response to a signal. It is a physiologic process useful to many organisms and an important counterpart to mitosis for the regulation of cell numbers during development and for homeostatic turnover in the adult. Usually, there is a balance between apoptosis and cell proliferation. Every change in this balance, on one or the other side, can lead to a pathological state.

#### *Physiological apoptosis*

Physiological mechanisms of cell elimination are required throughout the development and adulthood of all multicellular organisms. Cell-death events are essential to remove superfluous cells as a vital part of tissue sculpting during development, to maintain cellular homeostasis in developed adults and to remove harmful cells or cells with severe cellular or genomic damage from the organism (2). In the development of the central nervous system during which the brain undergoes physiological elimination of redundant neurones, the role of apoptosis is now proven. Developmentally occurring apoptosis affects more than 50% of human neurons and 0,5-1% of rodents neurons (3).

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### *Pathological apoptosis*

Apoptosis is also involved in some pathological processes. In some cases, apoptosis is inhibited and the inhibition of apoptosis may possibly lead to neoplastic cell proliferation (i.e. cancers (lymphoma, melanoma...)) or to autoimmune diseases by proliferation of self-directed lymphocytes. In other cases, apoptosis is hyperactivated (i.e. neurodegenerative diseases (Huntington's disease, Parkinson's disease)).

YOUNG C. *et al.* showed that the primary response to hypoxia/ischemia in the developing rodent brain is an excitotoxic neurodegeneration. This is followed by a delayed apoptotic response whereby neurons deafferented by the primary wave of excitotoxic cell loss commit suicide (4).

Finally there is evidence that apoptosis is involved in the fetal alcohol syndrome (1).

### *Morphological characteristics*

The term for physiological cell death, apoptosis, was introduced in the 1970s by KERR and colleagues to define a type of cell death distinct from necrosis, based on unique morphological characteristics (2). Apoptosis is characterized by a number of distinct morphological alterations of the cell such as internucleosomal DNA fragmentation, chromatin condensation, cell shrinkage and plasma membrane blebbing as well as cell surface alterations. These alterations are the signal for the rapid recognition and phagocytosis of apoptotic cells by the macrophages.

### *Triggers of apoptosis*

Apoptosis can be triggered by loss of growth factor, secondary signals due to DNA lesions, hormonal signals, hypoxia or tumour necrosis factor.

### *Molecules and pathways of apoptosis*

#### Caspases

Caspases are proteases that seem to constitute a complex proteolytic system, like the coagulation or complement cascade (2).

Activated caspases activate other members of the caspase family and proteolysis of diverse cellular proteins, all of which contribute to the controlled collapse of the cell.

They are synthesized as inactive proenzymes which need proteolysis for full catalytic activity (2).

Active caspases are responsible for producing the typical morphological changes associated with

apoptosis through targeting several proteins within the cell for activation or inactivation.

#### The mitochondrial pathway to caspase activation (or intrinsic pathway) (Fig. 1)

Mitochondria are an important sensor of cellular damage. Various forms of cellular stress such as DNA damage, heat shock and oxidative stress increase the permeability of the outer mitochondrial membrane.

This allows some proteins, such as cytochrome c, to be released from the mitochondrial intermembrane space into the cytosol. In the cytosol, cytochrome c binds to a protein called Apaf-1 (apoptotic protease activating factor-1). This binding results in a conformational change in Apaf-1 allowing its oligomerization.

At the mitochondrial level Procaspase-9 molecules can then bind to each of the Apaf-1 monomers.

This high molecular-weight complex is called apoptosome. Its formation promotes caspase-9 activation.

After activation within the apoptosome, caspase-9 propagates the caspase cascade through activation of caspases-3 and -7. In turn, caspase-3 activates caspase-2 and -6 and the latter promotes activation of caspases-8 and -10.

Caspase-3 is also involved in a feed-back amplification loop to further process caspase-9.

The activation of the caspase system will activate some nucleases which will destroy DNA.

#### Ligation of death receptors (or extrinsic pathway)

The death receptors are a subset of the TNF/NGF receptor family that contain a conserved motif, called the death domain (DD), within their cytoplasmic tails. These death domains are responsible for recruiting adaptor molecules that activates caspases.

In most cases, the recruited caspase is caspase-8 which can activate caspase-3 and then further disseminate the caspase cascade or activate other proteins that will engage the mitochondrial pathway by stimulating cytochrome c release which will activate caspases.

Extracellular signals such as members of the tumour necrosis factor (TNF) family can activate the receptor-mediated extrinsic pathway. The extrinsic pathway is initiated by the activation of death receptors that involves the formation of a death-inducing signalling complex (DISC). DISC formation results in the activation of Caspase-8, which activates caspase-3, executing the cell (5).

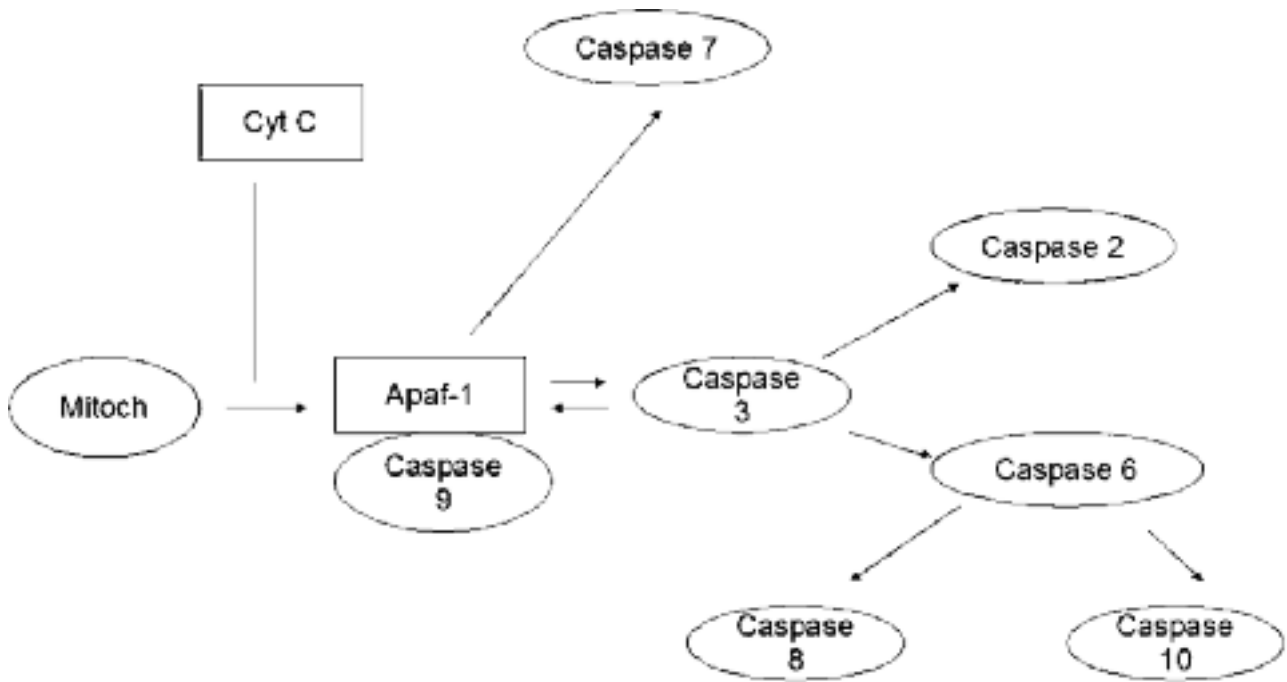


Fig. 1. — Caspase activation in the intrinsic pathway

### Bcl-2 family proteins

The Bcl-2 family features both anti-apoptosis and pro-apoptosis proteins. In the mitochondrial pathway, they are probably the most important regulators. They may be responsible for bridging signals from the death-receptor pathway to the mitochondrial pathway.

The Bcl-2 family proteins can interact with each other and also with several other proteins.

The most common type of interaction occurs between the antideath and prodeath members, such as Bcl-2 versus Bax (the first pro-apoptosis Bcl-2 family protein). This interaction can result in antagonistic action of the two types of molecules.

Because of the effects of Bcl-2 family proteins on the balance of life and death, cells impose strict regulations on the expression and activity of these molecules. Whereas some antideath or prodeath molecules are expressed constitutively in cells, others are expressed only following death stimuli.

The activation of the mitochondrial pathway is signified by the release of mitochondrial apoptotic proteins and by mitochondrial dysfunction.

The release of mitochondrial apoptotic proteins is due to an increase in outer membrane permeability, which may be provoked by the opening

of the mitochondria permeability transition pore of the pore formed by the Bcl-2 family proteins, such as Bax, or a pore made of components of both.

Interaction between caspases and Bcl-2 family proteins (Fig. 2)

Anti-apoptotic Bcl-2 family members such as Bcl-2 prevent cytochrome c release. Thus, they block the downstream intrinsic pathway events of apoptosome formation and caspase activation.

These anti-apoptotic effects of Bcl-2 proteins are countered by the pro-apoptotic actions of Bax on the apoptosome formation. Then, the modulation of apoptosome formation is a balance between pro-apoptotic and anti-apoptotic signals.

### Synthesis

Apoptosis is an evolutionarily conserved, genetically controlled process of programmed cell death, used by multicellular organisms to eliminate cells in diverse physiological settings, such as development, homeostasis of tissues and maintenance of integrity of the organism (6). Unfortunately, it can also be involved in some pathological processes such as the Fetal Alcohol Syndrome.

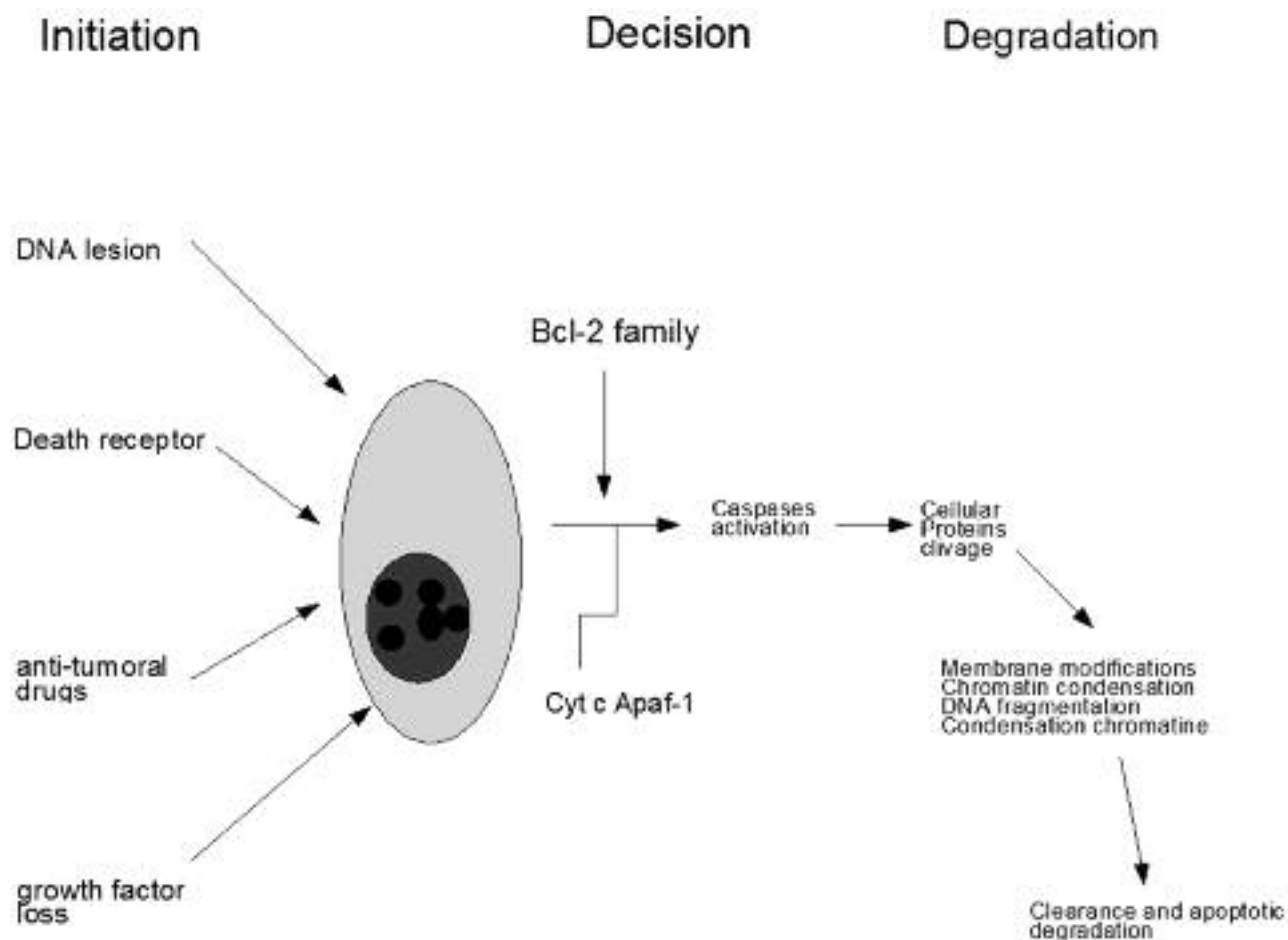


Fig. 2. — Interaction between caspases and Bcl-2 family proteins

It is interesting to develop the Fetal Alcohol Syndrome because of the similitude with the possible effects of general anesthetics on the developing brain.

#### PATHOLOGICAL CEREBRAL APOPTOSIS : THE FETAL ALCOHOL SYNDROME

Intrauterine exposure of the human fetus to ethanol causes a dysmorphogenic neuropathological syndrome that includes craniofacial malformation and a reduced brain mass. This is associated with a variety of neurobehavioral disturbances, ranging from hyperactivity/attention deficit disorder and learning disabilities in childhood, to major depressive and psychotic disorders in adulthood.

The fetotoxic effects of ethanol can lead to a partial syndrome including neurobehavioral disturbances ranging from mild to severe, unaccompanied by craniofacial dysmorphogenesis. This is called Alcohol-Related Neurodevelopmental Disorder (ARND) (1). Ethanol has both NMDA antagonist (7-8) and GABA (gamma-aminobutyric

acid) mimetic properties (9). It triggers a neurodegenerative response (10). The response is apoptotic (11), Bax-dependent (9) and involves activation of caspase-3 (12-13), which are characteristic features of apoptotic neurodegeneration.

Both rats (10) and mice (14) show a robust apoptotic response to ethanol.

In rodents, the window of vulnerability to ethanol-induced apoptosis coincides with the period of synaptogenesis, also known as the brain growth spurt period (1<sup>st</sup> to 14<sup>th</sup> days after birth). In the human, the synaptogenesis period spans the last three months of pregnancy and extends into the first years of life (15-16). In addition, there is a second period of accelerated synaptogenesis that occurs during adolescence, and there are some evidences suggesting an increased neurological vulnerability to the effects of alcohol and some drugs used recreationally during this time period (17).

In rodents, a single dose of ethanol is enough to cause many millions of neurons to commit suicide in the developing brain. In the most severely affected brain regions, the magnitude of cell loss

following a high dose of ethanol (5 g kg<sup>-1</sup> sc) is in the range of 45-68% (14).

A single ethanol intoxication episode (blood ethanol concentration > 200 mg/dl for 4 hours) can lead to apoptosis in the developing brain at the level of the hippocampus, thalamic nuclei (10-14), retina, visual cortex (18) and auditory cortex. Moreover, a severe single ethanol intoxication episode during the brain growth spurt period in rats does cause a significant reduction in brain mass. Thus, widespread and heavy loss of neurons from many brain regions during development results in an overall reduction in brain mass (10).

Many of these brain areas are involved in learning, memory, sensory information processing and cognitive function. Thus, it would be surprising if loss of neurons in these areas did not induce mental retardation in the ethanol toxic syndrome (1).

Moreover, all researchers who have examined the brain of Fetal Alcohol Syndrome patients, either at autopsy or by neuroimaging methods, have described a reduction in brain mass and dysgenesis or agenesis of the corpus callosum as findings that typify this syndrome (19, 20, 21-22).

In mice, ethanol reduces the corpus callosum to a fraction of its normal size (14).

In contrast, in utero exposure of rats to high doses of ethanol throughout gestation (before the growth spurt period) has no effect on the corpus callosum (23). This finding confirms that the effect on the corpus callosum during the brain growth spurt period is ethanol-induced and that there is a vulnerability period at this time. Another finding described in the Fetal Alcohol Syndrome neuroimaging literature (21-24), is a reduction in the size of basal ganglia, a term that refers to the caudate, putamen (lenticular nucleus) and anterior thalamus.

It was found that these brain regions are severely damaged in rats and mice following ethanol administration at any age from postnatal day 4 to 10 (10-14).

Interestingly, caudate neurons are exceedingly sensitive to ethanol and respond to even low doses with a robust display of caspase-3 activation at 3-4 h following a single subcutaneous injection of ethanol (13, 18-25). All these findings define the human Fetal Alcohol Syndrome as a good model of cerebral apoptosis for which experiments with rodents provide relevant information.

*By what mechanism does ethanol ingestion cause developing neurons to undergo apoptosis?*

The ethanol's apoptogenic activity is mediated by a combination of a NMDA antagonist and excessive GABA (GABA-A) mimetic mechanisms.

In genetically normal mice, it has been found (13) that ethanol triggers caspase-3 activation in neurons throughout the various brain regions where ethanol-induced apoptotic neurodegeneration has been demonstrated but it does not cause activation of caspase-8. This suggests an involvement of the intrinsic pathway rather than the extrinsic pathway in ethanol-induced apoptosis.

Another finding implicating the intrinsic mitochondrial pathway is that ethanol does not cause either caspase-3 activation or apoptotic cell death in the absence of the Bax gene (i.e. In homozygous Bax Knock-out mice) (12-26). Thus, it appears that ethanol-induced apoptotic neurodegeneration in the developing brain involves an action of Bax on mitochondrial membranes, causing abnormal release of cytochrome c and activation of the effector caspase-3 which executes a series of steps whereby the cell disassembles.

#### CEREBRAL DEVELOPMENT

Neurotransmitters are primarily considered as the effectors of synaptic transmission but, it is important to note that they are also present in the environment of neural cells in the early stages of central nervous system development (27-28) where they act as neurotrophic substances (29-30).

Their effects seem to be, at least partially, mediated through a paracrine diffuse mode of action where GABA and glutamate are non-synaptically released into the early differentiating cortical environment and activate their ionotropic receptors (30).

This might possibly mediate a wide range of developmental effects, including proliferation, differentiation and synapse formation (31).

For example, GABA was shown to have chemotactic effects during brain maturation by guiding the migration of newly generated neurons from the ventricular zone to the cortical plate (32-33). Glutamate, acting through the NMDA receptor, also stimulates embryonic cortical neuronal migration (32-34).

While endogenous GABA and glutamate are clearly key factors guiding central nervous system morphogenesis (35, 36, 37-38), exogenous stimulation or blockade of GABAergic and glutaminergic signalling pathways can also trigger cell death in the developing brain (37). Most anesthetic agents interact with GABA and NMDA receptors. Then the question arises whether they could trigger apoptosis in the developing brain.

PATHOLOGICAL CEREBRAL APOPTOSIS AND GENERAL ANESTHETIC AGENTS

Based on the action of ethanol as both NMDA antagonist and GABA agonist and its consequences on the brain, it has been hypothesised that drugs with NMDA or GABA effects could lead to pathological cerebral apoptosis.

Even if the exact mechanism of general anesthesia is not entirely understood, alteration of synaptic transmission involving gamma-aminobutyric acid type A (GABA A) and N-methyl-D-aspartate (NMDA) glutamate receptors, to different degrees, seem to play an important role (39).

Anesthetic drugs acting as N-methyl-D-aspartate (NMDA) receptor antagonists and those acting in an agonistic manner at the gamma-

aminobutyric acid (GABA) receptor include the entire classes of barbiturates (GABA), benzodiazepines (GABA) and halogenated anesthetics, as well as chloral hydrate (GABA), etomidate (GABA), propofol (GABA-NMDA), ketamine (NMDA) and nitrous oxide (NMDA and GABA) (38).

So, most of anaesthetic drugs have the possibility to induce cerebral apoptosis.

#### *GABA agonist drugs*

IKONOMIDOU C. *et al.* (10) studied, in rats, different drugs such as: diazepam (10-30 mg kg<sup>-1</sup> IP), clonazepam (0,5-4 mg kg<sup>-1</sup>), pentobarbital (10 mg kg<sup>-1</sup>), phenobarbital (50-75 mg kg<sup>-1</sup>). All these agents triggered widespread apoptotic cell death in the infant rat brain. The pattern of degeneration observed in rat brain (cerebral areas where widespread cell death took place) was similar for each GABAergic agent but differed in several respects from that induced by NMDA antagonists. However, the superimposition of one pattern on the other resulted in a composite pattern closely similar to that induced by ethanol. In this study, the window of vulnerability for the proapoptotic actions of diazepam and phenobarbital coincides with the period of synaptogenesis.

Recent observations suggest that midazolam can also induce apoptosis in the immature central nervous system (40). In this study, 7-day-old mice were given a single subcutaneous dose of midazolam (9 mg kg<sup>-1</sup>). The authors demonstrated that this treatment was insufficient to induce anesthesia but induced a significant neuroapoptotic response in the cerebral cortex as well as in the basal ganglia.

#### *Inhaled anesthetics and nitrous oxide*

All volatile agents have GABA mimetic and/or NMDA antagonist properties (31). For example, nitrous oxide is a potent antagonist of the NMDA type of glutamate receptor (31).

JEVTOVIC-TODOROVIC and colleagues (41) anesthetized neonatal rats on the seventh day postnatally with combinations of isoflurane, nitrous oxide and midazolam (a combination of drugs commonly used for long pediatric surgical procedures) sufficient to maintain a surgical plane of anesthesia for 6 hours. Histopathology showed that isoflurane (0,75-1,5%) caused a dose-dependent increase in apoptotic neurodegeneration. Midazolam (3-9 mg kg<sup>-1</sup>) and N<sub>2</sub>O (50-150% in a hyperbaric chamber) when given alone did not increase apoptosis compared to control animals. However, midazolam followed by maintenance with isoflurane (double cocktail) increases the damage caused by isoflurane alone, primarily in the thalamus and the parietal cortex.

The damage was even worse when N<sub>2</sub>O was added during the maintenance phase of anesthesia (by using: midazolam 9 mg/kg, isoflurane 0,75%, N<sub>2</sub>O 75%). The triple cocktail composed by midazolam, isoflurane and nitrous oxide caused widespread neuron loss with a more than 15-fold increase in the number of apoptotic neurons.

Animals exposed to the triple cocktail during their neonatal period had measurable deficits in spatial memory tests and demonstrated persistent learning impairments later in life.

Recently, isoflurane neurotoxicity was tested in cultured slices from rat brain. This «in vitro» model has the advantage of eliminating possible confounding factors present «in vivo» such as hypoxemia, acidosis, hypoglycemia, etc. Isoflurane 1,5% for 5 hours causes a significant increase in the number of apoptotic neurons in slices obtained from 7-day-old animals. Cultures obtained from younger and older animals or exposed to isoflurane for shorter time periods were less affected (42).

Preliminary results from guinea pigs (synaptogenesis in this species takes place prenatally and over a much longer time period than in rats) (43) and from piglets (where synaptogenesis is prolonged and spans pre- and postnatal time periods) (44) show that these animals are also susceptible to anesthetic neurotoxicity if exposed to midazolam, isoflurane and nitrous oxide pre- (guinea pigs) and postnatally (piglets).

In a recent study, JOHNSON S. A. and colleagues concluded that exposure to sub-MAC concentrations

of isoflurane for one or more hours triggers neuroapoptosis in the infant mouse brain (45). In these experiments, infant mice were exposed to isoflurane at various sub-MAC concentrations and durations, and the brains were evaluated quantitatively 5 hours after initiation of anesthesia exposure to determine the number of neuronal profiles undergoing apoptosis. Blood glucose values were also determined under each of these conditions. All conditions tested (isoflurane at 0.75% for 4 h, 1.5% for 2 h, 2.0% for 1 h) triggered a statistically significant increase in neuroapoptosis compared with the rate of spontaneous apoptosis in littermate controls. Blood glucose determinations ruled out hypoglycemia as a potential cause of the brain damage.

Li Y and colleagues investigated the behavioural and neurotoxic effects of fetal exposure to isoflurane in a maternal fetal rat model. Pregnant rats at gestational day 21 were anesthetized with 1.3% isoflurane for 6 h. Apoptosis was quantified in the hippocampus and cortex at 2 and 18 h after exposure in the fetal brain and in the postnatal day 5 (P5) pup brain. Spatial memory and learning of the fetal exposed pups were examined with the Morris Water Maze at juvenile and adult ages. Rat fetal exposure to isoflurane at pregnancy day 21 through maternal anesthesia significantly decreased spontaneous apoptosis in the hippocampal CA1 region and in the retrosplenial cortex at 2 h after exposure, but not at 18 h or at P5. Fetal exposure to isoflurane did not impair subsequent juvenile or adult postnatal spatial reference memory and learning and, in fact, improved spatial memory in the juvenile rat. These results show that isoflurane exposure during late pregnancy is not neurotoxic to the fetal brain and does not impair memory and learning in the juvenile or adult rat (46).

### *Ketamine*

Ketamine was the most studied anesthetic drug in the field of neuroapoptosis because it was first used in the field of cerebral neuroprotection.

HAYASHI *et al.* (47) showed that a single intraperitoneal dose of ketamine (25, 50 and 75 mg kg<sup>-1</sup>) did not increase neurodegeneration in rat compared with the control group.

However, repeated doses of ketamine (25 mg kg<sup>-1</sup>) at 90 minutes intervals over 9 hours increased degenerating neurones in seven out of 10 examined brain regions (Temporal cortex, hippocampus CA1, dentate gyrus, thalamus laterodorsal, mediodorsal and ventromedial, basomedial amygdala where

affected while the layer II of the retrosplenial, parietal cortices and ventromedial hypothalamus were not). They suggest that the duration of ketamine exposure correlates with increased neuronal degeneration in the developing rat brain.

FREDRIKSSON *et al.* (48) demonstrated that a single dose of ketamine administered to a 10-day-old mouse induced a significant increase in cellular degeneration in the parietal cortex. The combination of diazepam and ketamine resulted in greater neuronal toxicity than ketamine alone even if diazepam did not enhance the ketamine-induced functional deficits (deficits of acquisition learning and retention memory) measured up to months after injection.

YOUNG *et al.* (49) noted that a single sub-anesthetic dose of ketamine triggers a fourfold increase in neuroapoptosis in the infant rodent brain.

«In vitro» studies demonstrated cell death in neurones cultured from the rat forebrain after prolonged exposure to ketamine at concentrations of 10 or 20  $\mu$ M. However, no cell death was evident after exposure to ketamine concentrations of 0.1 or 1.0  $\mu$ M (50).

Studies conducted with cultured monkey frontal cortical neurons produced the same results (51). SLIKKER *et al.* (52) recently reported their findings of ketamine-induced neurotoxicity in prenatal rhesus monkeys after IV infusions of ketamine administered to pregnant females at doses sufficient to maintain a steady-state anesthetic plane for 24 hours on gestational day 122, followed by a 6-hour washout period. Examination of tissue isolated from the frontal cortex of the monkey fetus showed enhanced cell death consistent with an apoptotic mechanism. In this study, they suggested a differential pattern of damage in the prenatal monkey brain compared with that seen in the neonatal rat because only a few degenerating neurons could be identified in the ketamine-treated fetal monkey hippocampus, thalamus, basal ganglia, hypothalamus or amygdala. In ketamine-treated monkeys, extensive neuronal cell death occurred in the superficial neocortical layers and in the allocortex.

Finally, small cells immunopositive for proliferating cell nuclear antigen were also observed in the superficial neocortical layers of the ketamine-treated monkeys, suggesting the possibility of some compensation for the neuronal loss (53).

Whether the observed cell death affects overall brain function, or whether the injured brain tissue can recover with no loss of normal function, has still to be investigated.

### *Propofol*

Propofol interacts with both GABA A receptors and NMDA glutamate receptors (54-55).

It reportedly causes neurodegeneration and neurocognitive disturbances when administered together with ketamine to infant mice (56).

In a recent study, CATTANO and colleagues first determined the intraperitoneal dose of propofol required to induce surgical plane of anesthesia in the infant (5-7 days) mouse. The required dose was 200 mg/kg. They then administered graduated doses of propofol (25-300 mg/kg) and found that doses  $\geq$  50 mg/kg induce a significant neuroapoptosis response (57).

### *Combined use of anesthetics*

A large body of experimental evidence suggests that concurrent use of several anesthetics can potentiate cerebrocortical damage (31). Co-administration of even low doses of ketamine and nitrous oxide in rats enhances neurotoxic reaction to a much greater degree that can be explained by simple additivity between these agents (58).

Recently, co-administration of sedative concentrations of midazolam and ketamine to the infant mouse brain has been shown to be more effective in inducing apoptosis than either of these drugs alone (40).

Finally, exposure of 7-day-old rats to a combined midazolam-nitrous oxide-isoflurane anesthesia for 6h induced widespread neurodegeneration in the developing brain and this was accompanied by persistent learning deficits (41).

Fredriksson A and colleagues concluded in a recent study that the combination of a gamma-aminobutyric acid type A agonist (thiopental or propofol) and a N-methyl-D-aspartate antagonist (ketamine) during the brain growth spurt potentiated neonatal brain cell death and resulted in functional deficits in adulthood in rodents. In this study, the use of thiopental, propofol and ketamine individually elicited nor or only minor changes (56).

### *Anesthetics activation of apoptotic pathways*

Recently, it was proposed that anesthetics activate both the intrinsic and extrinsic apoptotic pathways, depending on the length of the exposure (5).

In neonatal rats, the intrinsic pathway is activated within 2 h of anesthesia exposure, while activation of the extrinsic pathway occurs within 6 h (5).

### IS IT POSSIBLE TO PREVENT OR DIMINISH PATHOLOGICAL CEREBRAL APOPTOSIS ?

Administration of  $\beta$ -estradiol has been shown to reduce anesthesia-induced apoptosis in the developing brain (59).

The apoptotic neurodegeneration induced by the midazolam-nitrous oxide-isoflurane "cocktail" has been reported to be dose-dependently reduced by the administration of melatonin (60).

In a recent study, piglets aged 2-5 days were exposed to hypoxia but treated by clonidine (12,5 mg kg<sup>-1</sup>) over 30 min before induction of hypoxia. It shows that Apaf-1 (apoptotic protease activating factor-1) is expressed during hypoxia in the cerebral cortex of newborn piglets and that administration of clonidine prevents the hypoxia-induced increased expression of Apaf-1 (6).

Several lines of evidence suggest that, under certain conditions, hypoxia induces cell death through the Apaf-1 mediated mitochondrial pathway (6). Other drugs that have been given to humans without apparent toxicity may have efficacy against underlying apoptotic mechanisms (minocycline, lithium) (61, 62, 63, 64).

In primary cultures of rat cerebellar granule cells and cortical neurons, Chuang DM showed that lithium potently protect against glutamate-induced, N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity. The neuroprotective mechanisms involve induction of neurotrophic/neuroprotective proteins including Bcl-2 (63). In a rat cerebral artery occlusion model of stroke, postinsult treatment with lithium reduces ischemia-induced brain infarction, caspase-3 activation and neurologic deficits (63).

Finally, in a rat excitotoxic model of Huntington's disease in which an excitotoxin is infused into the striatum to activate NMDA receptors, short-term lithium pretreatment is sufficient to protect against DNA damage, caspase activation, and apoptosis of striatal neurons, and this neuroprotection is concurrent with Bcl-2 induction (63).

In his study, Chen DM and colleagues (64) showed, in a glutamate-induced neurotoxicity model, that long-term treatment of cultured cerebellar granule cells with LiCl induces a concentration-dependent decrease in mRNA and protein levels of proapoptotic Bax ; conversely, mRNA and protein levels of cytoprotective Bcl-2 are increased. Lithium pretreatment also blocks glutamate-induced cytochrome c release (64).

Interestingly, in one animal study, the NMDA-antagonist xenon, did not cause neurotoxicity when administered at 0.5 MAC for 6 h. Xenon has also

shown potential for mitigating isoflurane-induced neuronal degeneration when administered in combination with isoflurane (65).

Insulin-like growth factor (IGF-1) given to immature rats of lambs after hypoxia-ischemia significantly reduces the severity of brain damage and is associated with reduced caspase-3 and caspase-9 activity (66-67). The finding that IGF-1 remains effective when given up to 6 h posthypoxia suggests that IGF-1 acts on the phase of delayed neuronal loss.

As caspase are effectors of apoptotic cell death, caspase inhibitors may help to preserve neuronal function by extending the therapeutic window and providing long-term neuroprotection. Recently, a specific caspase inhibitor was shown to prevent neonatal stroke in rat (68). These encouraging observations open a whole new line of research aimed at counteracting anaesthesia-induced neurotoxicity and further studies should be conducted to address this issue.

#### THE ROLE OF PAIN

Anesthesia in rodent studies is usually administered without noxious stimulation but, during pediatric surgical anesthesia, the central nervous system is stimulated by surgical interventions and painful stimulation (69).

Recently, a study in a newborn rat model documented neurodegenerative effects and behavioral impairment after repetitive painful stimulation, which were ameliorated by a low dose of ketamine (5 mg kg<sup>-1</sup>) (70).

Furthermore, structural brain abnormalities and long-term behavioral abnormalities have been documented after painful stimulation in unanesthetized, newborn humans and animals (71).

#### ONLY THE BRAIN ?

SANDERS and colleagues (72) recently embarked on a study focusing on the spinal cord as possible site of injury secondary to anesthetic exposure to isoflurane and nitrous oxide.

Seven-day-old rat were exposed to 6 h of nitrous oxide (75%) + isoflurane (0,75%) in oxygen (25%) or air.

They determined that anesthesia with nitrous oxide-isoflurane for 6 h increased the number of caspase-3 positive cells relative to air exposure. The site expressing the biggest increase in caspase-3 reactive cells appeared to be within the ventral horn

of the spinal cord, although dorsal horn neurons were also affected.

However, motor responses were not affected by neonatal anesthetic treatment.

#### CONCLUSION

Cerebral apoptosis is an important physiological process by which redundant neurons are eliminated. It involves many complex intracellular and molecular processes.

During periods of synaptogenesis, general anesthetics can induce excessive cerebral apoptosis (histopathologic changes) in juvenile animal models.

A similar phase of synaptogenesis does exist in humans. During this period human brain seems to be vulnerable to the action of some drugs such as ethanol.

To characterize the neurotoxic effects of drug exposure on the developing human brain, clinicians and scientists rely on experimental data collected from various animal species that develop and mature at varying rates.

Despite the accumulating animal data on anesthetic neurotoxicity, the limitations of the aforementioned experimental models preclude their direct applicability to the clinical care of humans.

This, coupled with evidence from clinical studies showing that acute and long-term effects of unrelieved pain or surgical stress, justify the clinical use of potent anesthetics for neonates and infants.

Future scientific experiments must be designed using animals at comparable neurodevelopmental stages, using clinically relevant doses and durations of anesthetic exposure to counteract the stimuli of some defined surgical procedures with adequate support guided by continuous physiologic monitoring. Finally, the association of locoregional with general anesthesia is increasingly used in modern anesthesia including for children. Animal research comparing neuronal apoptosis after general anesthesia alone vs. associated with a locoregional technique would be most welcome.

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