

Are General anesthetics neurotoxic?

REVIEW

Abstract

Introduction: General anaesthesia has been used worldwide since its first public demonstration with ether in 1846. Until a little more than a decade ago, it was believed that the anaesthetic state was limited to the period of exposure. Studies in rats, pigs, and rhesus monkeys have shown that almost all general anaesthetics accelerate the apoptotic process in neurones, oligodendrocytes, and glial cells.

Objective: Our aim was to analyse the relationship between general anaesthetics and their role in triggering neuroapoptosis in laboratory animals.

Method: A search was carried out in PubMed and Google Scholar with the keywords "*neurotoxicity*" and "*general anesthetics*" for selecting articles published in the last five years. After having evaluated the abstracts, 77 articles were selected and read by 2 independent investigators. All authors met and discussed the most relevant aspects.

Results: All general anaesthetics, when inhaled or administered intravenously, enhance neuroapoptosis, mainly during the gestational and neonatal stages in rats, pigs, and non-human primates. Neurones and oligodendrocytes that are capable of neurogenesis and synaptogenesis are the most affected. General anaesthetics commonly lead to learning and behavioural disorders, in addition to permanent memory deficit.

Conclusion: The neurotoxicity of general anaesthetics affects different mammalian species and accelerates the neuroapoptotic process. This deleterious effect involves specific brain areas and occurs in developing neurones. The exceptions are the dentate gyrus and the olfactory bulb, which undergo apoptosis even in adulthood, albeit to a lesser extent.

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Keywords

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Introduction

Although the anaesthetic properties of ether were discovered 150 years ago, its mechanism of action is still unknown. However, it has huge advantages allowing millions of surgical procedures under general anaesthesia to be performed every day, thus being considered one of the ten most important discoveries of mankind [1].

Even after all this time and with all the advances in science, what is known about its mechanisms of action is related to its ability to bind to the inhibitory GABA receptors, potentiating their action, as well as to the excitatory NMDA receptors, causing their inhibition. However, the mechanisms of action of these drugs in the neural networks and brain areas involved in the regulation of consciousness and anaesthesia [1] still need to be elucidated.

Thus, as the mode of action of general anaesthetics is poorly understood, further investigations are required to assess the adverse effects of these drugs. Several studies on animal models suggest that a permanent learning deficit and memory disorders may occur in the offspring of laboratory animals receiving propofol or isoflurane during pregnancy [10].

Microbiological and immunofluorescence assays showed increased caspase-3 levels as well as reduced levels of synaptophysin in the foetal brain—both being markers of increased apoptosis (programmed cell death)—when compared to laboratory animals that were not exposed to propofol [2].

The neurotoxicity of general anaesthetics was inherently attributed to the foetal and neonatal periods of the animals, in which neurogenic processes are still observed. However, studies show that increased neural apoptosis may also occur in young, adult, and aged laboratory animals [3]. Animals at a more advanced stage of development present neuroapoptosis only in specific regions of the brain, albeit in lower intensity [12].

Several studies in animal models, in which brain sections of different regions were analysed by im-

munohistochemistry, showed an increase in apoptosis induced by general anaesthetics, when used at concentrations normally administered in daily medical practice. The apoptotic cell death induced by the anaesthetics increases with the concentration of the anaesthetic used and the time of exposure, besides being enhanced by ketamine and isoflurane [13, 26].

Neurons in the early stages of development are more susceptible and the window of vulnerability varies according to the brain region. During the foetal period, the cortex and caudoputamen are more susceptible. However, the greatest sensitivity to neuroapoptosis was observed during the neonatal period in the dentate gyrus. This region is responsible for learning, memory, and behavioural development, and undergoes synaptogenesis until adulthood [8-10].

Therefore, although general anaesthetics are used in medical clinics to carry out different procedures, the neurotoxic effects induced by their administration are still poorly understood and further investigations are required to minimize the adverse effects and ultimately lead to a lower number of complications.

Thus, this study aims to analyse the correlation between general anaesthetics and their effects in triggering neuroapoptosis in laboratory animals.

Method

This systematic review was conducted according to the following steps: establishment of the hypothesis and objectives of the review; establishment of sample selection based on criteria for the inclusion and exclusion of the articles; definition of the information to be extracted from the articles selected; analysis of the results; discussion and presentation of the results; and presentation of the review.

To guide the review, the following question was formulated: How are the neurotoxic effects of general anaesthetics investigated?

The articles were selected by a search carried out in PubMed/Medline and Google Scholar databases, considering only articles published in English in the last 5 years. The articles were searched using the keywords "Anaesthetic Neurotoxicity," and a total of 217 articles were found in PubMed (**Table 1**).

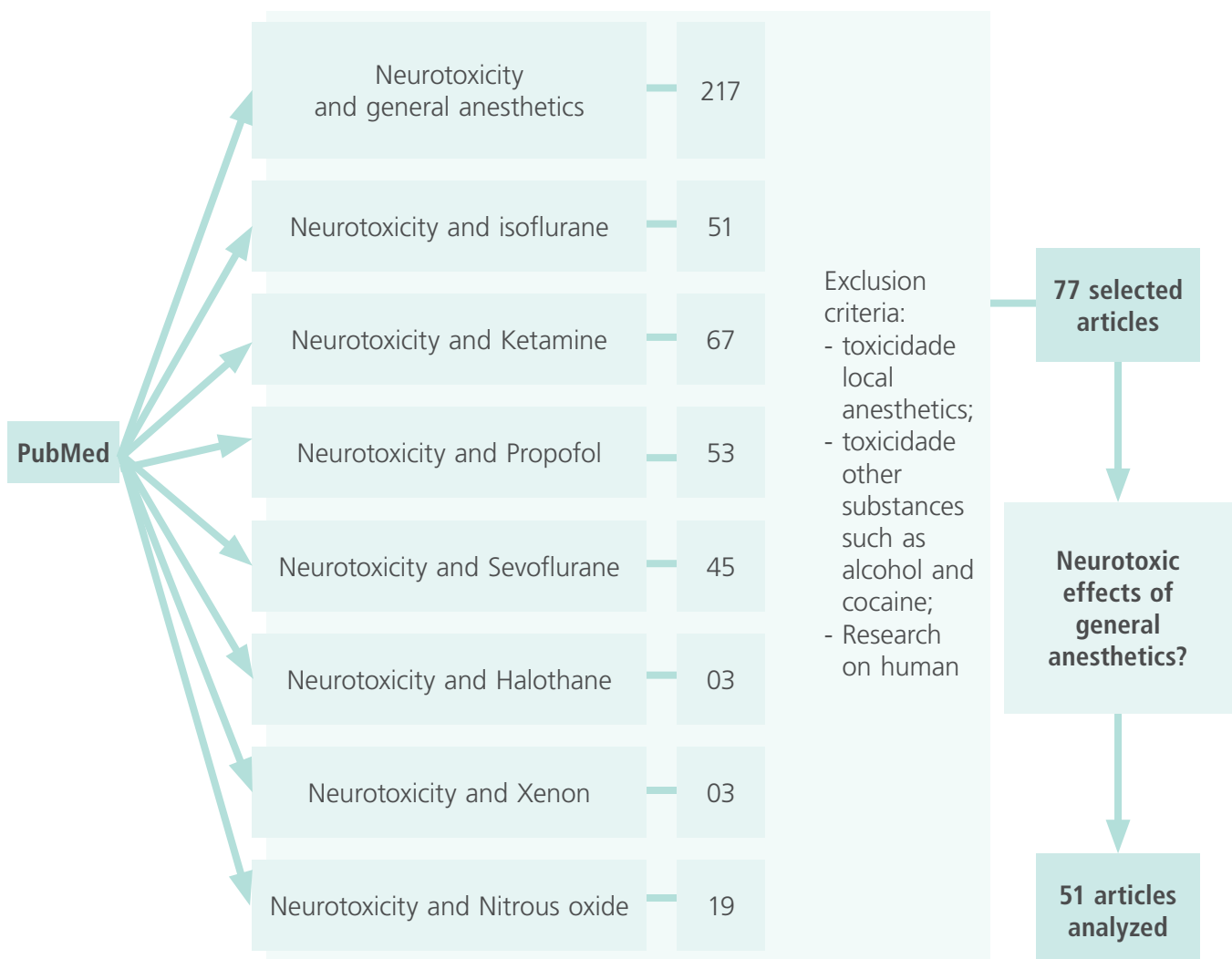
The articles referring to the toxicity of local anaesthetics or reporting the toxicity induced by other substances such as alcohol and cocaine were excluded along with the studies in humans. Seven-

ty-seven remaining articles were analysed by two independent investigators, who found 27 relevant papers. The results were then compiled and discussed with the other authors of this study.

Results

Most of the studies reported a significant increase of caspase 9 and 3, the main markers of cell apoptosis. Therefore, the triggering of the apoptotic cascade leading to cell death is associated with the

Table 1. Database search with keywords for articles published in English over the past 5 years. Articles referring to human studies or the use of other drugs, such as local anaesthetics and alcohol, were excluded. Of the 77 articles thus identified, 27 were considered relevant after a detailed analysis.



stimulation by anaesthetics of the GABA receptors, localized on the cell membrane, and the inositol triphosphate and ryanodine receptors localized on the endoplasmic reticulum. Increased levels of intracellular calcium were observed, which promote further release of calcium from the mitochondria. This results in the release of cytochrome c, which leads to the activation of caspases.

The analysed studies show that in laboratory animals (rats, pigs, and rhesus monkeys), there is an increased apoptosis of neural cells in foetuses and new-borns. Moreover, enhanced apoptosis was also observed in young and adult samples, in regions that were still undergoing neurogenesis, which is a slower process in this age group [10].

Isoflurane, propofol, sevoflurane, halothane, desflurane, nitrous oxide, and especially ketamine were already shown to increase apoptosis in newborn and young laboratory animals. However, no neurotoxic effects were presented by the alpha-2 agonists, dexmedetomidine and clonidine. One study demonstrated that the preoperative administration of clonidine inhibited neuroapoptosis in neonatal mice receiving ketamine [5].

Discussion

Neuroapoptosis is a physiological process of programmed cell death, which eliminates defective neurones. In the last decade, several studies addressed the beneficial and deleterious effects of anaesthetics in neurones and oligodendrocytes. The search for the mechanisms of action affording neuroprotection or neurotoxicity caused by these substances is currently ongoing [11].

Potential targets are GABAA and NMDA receptors, in addition to the promising presynaptic α 2-agonists represented by clonidine and dexmedetomidine. These act on noradrenergic receptors in the locus ceruleus, which is a site of action in the brain very different from other classes of anaesthetics regulating wakefulness and sleep status. In addition,

when administered intrathecally, these act on the pain signaling pathway via the spinal cholinergic system [11].

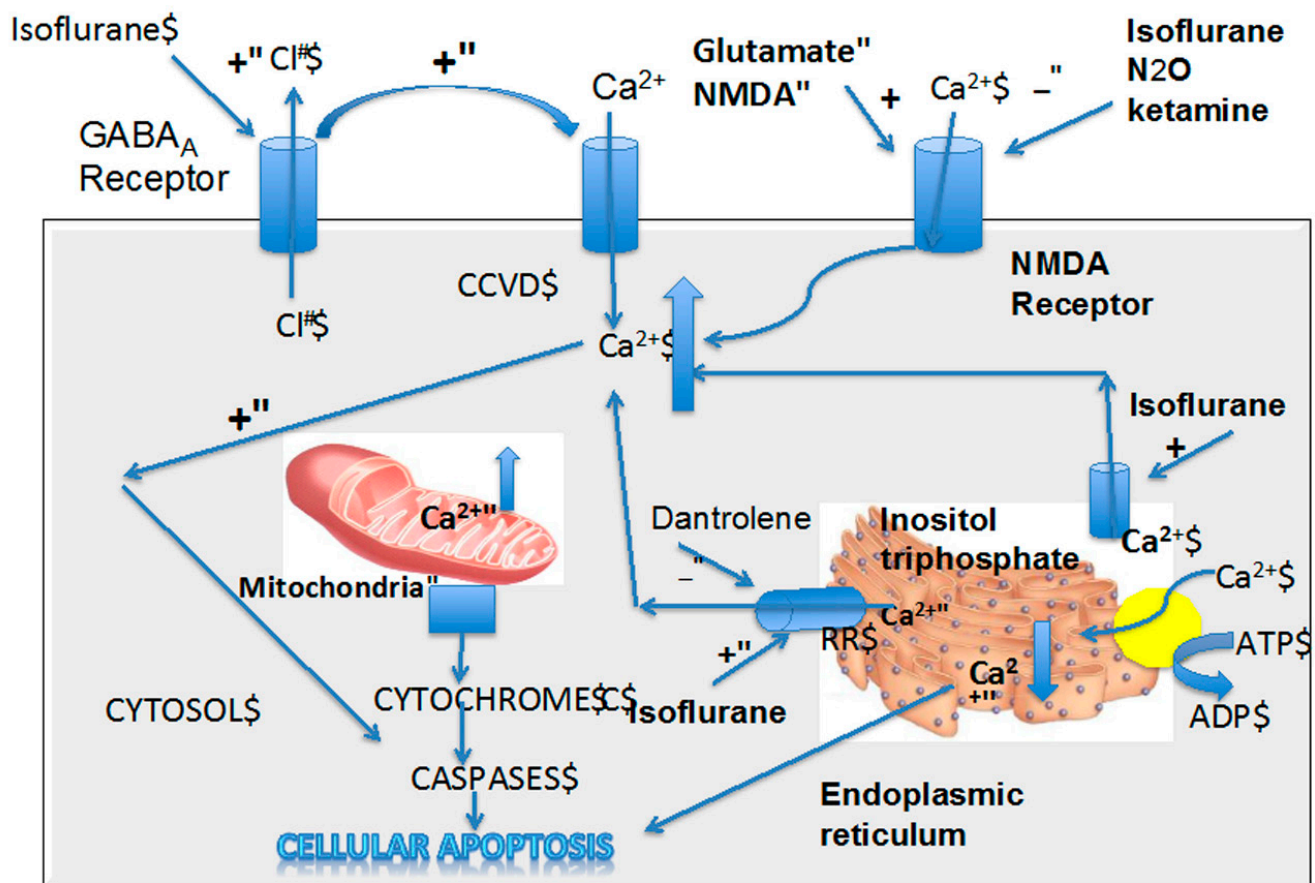
Practically all general anaesthetics, with the exception of α 2-agonists (dexmedetomidine and clonidine) [9], when inhaled or administered intravenously, trigger variable levels of apoptosis. This phenomenon relies on the dose, time, and number of exposures, besides the type of anaesthetic. Isoflurane and ketamine are associated with more severe brain injuries [14].

One of the most probable mechanisms involves the activation of voltage-dependent calcium channels, which allow the entry of calcium into the cytosol. The increase of intracellular calcium triggers further calcium release from mitochondrial storages. In the endoplasmic reticulum, isoflurane directly induces the opening of ryanodine and inositol triphosphate channels, thus further increasing the levels of calcium in the cytosol and triggering the activation of an enzyme named calpain. The release of cytochrome c then stimulates the activation of caspase 9 and 3, the main mediators of cellular apoptosis [8, 21] (**Figure 1**).

Previously, cognitive disorders, affecting mainly younger and older age groups, were considered temporary and reversible. It is well established in laboratory animals that exposure to general anaesthetics in the neonatal period leads to behavioural disorders and learning deficits that persist in adolescence and adulthood [12, 13, 17].

Neuroapoptosis is more prevalent in cells with increased neurogenic activity. This occurs at different periods of life and according to the brain region. In the cortex, thalamus, caudoputamen, and hippocampus, peak development occurs in the neonatal period. The activation of caspase 3 (the main marker of apoptotic cell death) observed in these regions was significantly higher in young rats derived from mothers that received isoflurane than in rats that were not exposed to the anaesthetic [15].

Figure 1: Proposed mechanism of neuroapoptosis of immature neurones triggered by general anaesthetics. Isoflurane activates GABA receptor channels in the cytoplasmic membrane as well as ryanodine and inositol triphosphate receptors in the endoplasmic reticulum. This increases the intracellular calcium influx and stimulates the release of calcium from mitochondrial calcium stores, which causes the release of cytochrome c and activation of caspase 9 and 3, thus resulting in neuronal apoptosis. Adapted from Wei, 2011.

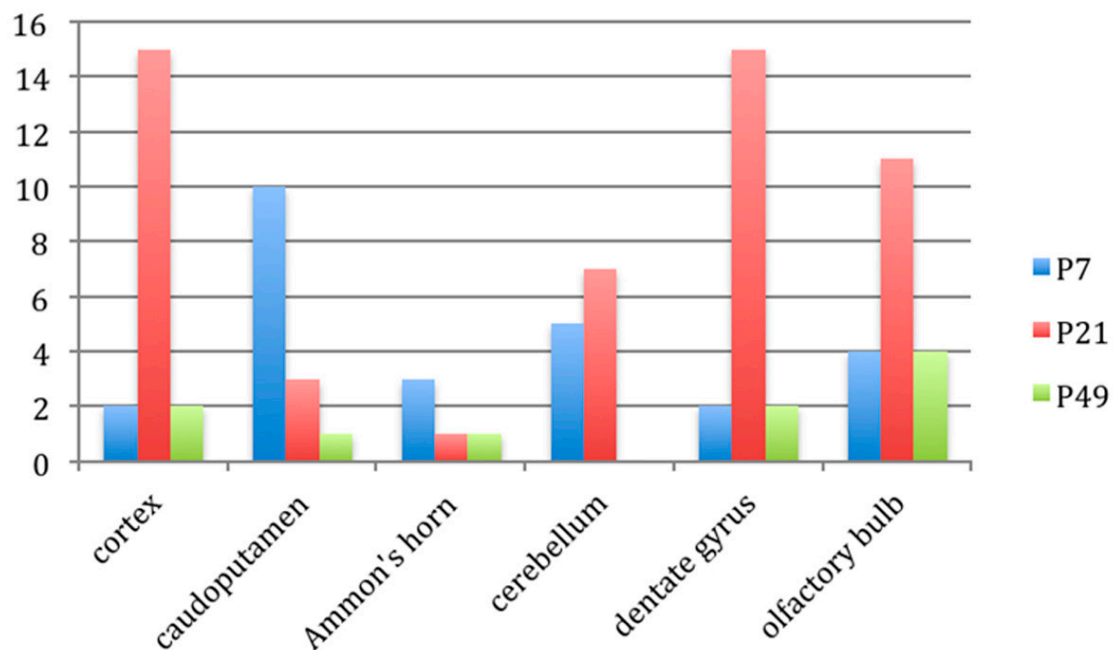


Creeley et al. studied the effects of neonatal brain exposure to propofol and isoflurane in rhesus monkeys by measuring the extent of apoptosis in neurones and oligodendrocytes. They found scattered areas of apoptosis in the group that did not receive any drugs, which was considered normal in this age group. In the group that received isoflurane, they noted areas showing densely concentrated apoptosis, affecting mainly the cortical layers II and V. The group that received propofol developed lesser laminar pattern degeneration, although it occurred in the same areas that were affected by isoflurane [20].

Not all areas of the brain are susceptible to neuroapoptosis caused by anaesthetics. There are windows of vulnerability, which vary according to the region and developmental stage of the neurones [16] (Figure 2).

Several research strategies have been used to find a solution to this potentially severe side effect of general anaesthetics. Pontén et al., in a study using rats, concluded that the administration of clonidine as a pre-anaesthetic medication suppresses the neuroapoptosis triggered by ketamine [5]. However, these promising data require further confirmation.

Figure 2: Window of vulnerability to the neuroapoptosis induced by isoflurane exposure, when used at a concentration of 1.5% and for over 6 hours. P7, P21, and P49 correspond to the age of the evaluated rats (newborn, young, and adult). The histograms correspond to the density of neuronal apoptosis. In P7, a marked increase is observed in the cortex and caudoputamen. Younger rats (P21) showed increased apoptosis in the dentate gyrus and olfactory bulb, which are regions susceptible to apoptosis, although less than in adult rats. Adapted from Deng, 2013.



Most of the evaluated studies focused on the neurotoxicity of isoflurane, ketamine, and propofol. Some studies assessed the effects of using more than one anaesthetic simultaneously and observed greater learning and spatial memory deficit with the administration of isoflurane. Higher

incidence of behavioural disorders was observed to be triggered by the use of ketamine, whereas learning, memory, attention, and spontaneous motor activity deficits were caused by the use of nitrous oxide, as shown in **Table 2**.

Table 2. Neurotoxicity induced by general anaesthetics in laboratory animals in the 27 selected articles. Several articles addressed the effects of more than one anaesthetic.

Compound	Number of studies	Effects <i>in vivo</i>
Isoflurane	10	Learning disorders, spatial memory deficit, attention and spontaneous motor activity deficit
Sevoflurane	7	Learning and social memory disorder
Propofol	8	Spontaneous activity deficit, learning disorders, and spatial memory deficit
Thiopental	3	Behavioural disorders
Nitrous oxide	6	Learning, memory, attention and spontaneous motor activity deficit
Midazolam	4	Learning, memory, attention and spontaneous motor activity deficit
Ketamine	13	Behavioural disorders

Conclusion

Halothane, isoflurane, sevoflurane, desflurane, nitrous oxide, xenon, propofol, and ketamine were shown to trigger neuroapoptosis. The studies demonstrated that ketamine and isoflurane have a greater potential to induce neuronal death. The administration of clonidine suppressed neuroapoptosis in rats that received ketamine, thus suggesting a possible protective action of clonidine.

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