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Anaesthetic-induced developmental neurotoxicity on (neuro) steroids

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This editorial accompanies the following articles:

Neuroactive steroids alphaxalone and CDNC24 are effective hypnotics and potentiators of GABAA currents, but are not neurotoxic to the developing rat brain by Tesic et al., *Br J Anaesth* 2020:124:603–13, doi: 10.1016/j.bja.2020.01.013

The T-type calcium channel isoform Cav3.1 is a target for the hypnotic effect of the anaesthetic neurosteroid (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile by Timic Stamenic et al., *Br J Anaesth* 2021:126:245–255, doi: 10.1016/j.bja.2020.07.022

Keywords: alphaxalone; anaesthetic mechanism; apoptosis; calcium channel; gamma-aminobutyric acid; neurosteroid; neurotoxicity

Two decades ago, Jevtovic-Todorovic and colleagues[1] published the seminal observation that neonatal rodents developed neuroapoptosis and long-term cognitive deficits after administration of a mixture of general anaesthetics including midazolam, nitrous oxide, and isoflurane. Subsequent work has confirmed these findings in rodents and extended them to include most of the currently used anaesthetics.[2] The neurotoxic effects of anaesthetics appear to be dependent on the developmental time point of administration, number of exposures, and dose and duration of each exposure.[3,4] Most importantly, studies in non-human primates in which physiological and biochemical parameters can be closely monitored confirm the long-term behavioural changes associated with neonatal anaesthetic administration and do not identify a confounding physiologic variable (i.e. hypotension or hypoxaemia) as the cause of neurotoxicity.[4,5] The results of these animal model studies led the US Food and Drug Administration (FDA) to place a 'black-box' safety warning on a long list of anaesthetic agents regarding their use in neonates.

Human data have been more complex with several epidemiologic studies showing that prolonged and repeated anaesthetic exposures are associated with persistent behavioural changes,[6,7] but prospective trials showing that single brief exposures produce no discernible effect.[8,9] Unfortunately, the critical question of whether long or repeated general anaesthetic exposures are neurotoxic in human neonates is difficult to address, since infants needing general anaesthesia often have underlying conditions and require surgeries that can predispose to cognitive deficits. Efforts to address neurotoxic effects of prolonged and repeated human neonatal exposure to general anaesthesia have focused on the approach of identifying either a non-neurotoxic anaesthetic or a mitigating (neuroprotective) agent that could be used in clinical trials as a comparator to currently used agents.

The search for a non-toxic anaesthetic or mitigating agent would be facilitated by understanding the underlying biochemical mechanisms of anaesthetic-induced developmental neurotoxicity (AIDN) in animals. Regrettably, there is no consensus on mechanism or even whether neuronal apoptosis is causal of the persistent cognitive/behavioural deficits.[10] It is hypothesised that anaesthetic effects on synaptic activity at a critical time in nervous system development promote apoptotic death of neurones and glia, and loss of synaptic connectivity. Since most anaesthetics produce their anaesthetic effect by activating postsynaptic gamma-aminobutyric acid A ($GABA_A$) receptors or blocking *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, these two neurotransmitter receptors have been a major focus of investigation.

Neurosteroids are endogenous brain metabolites of cholesterol that modulate inhibitory neuronal tone and are thought to act as endogenous regulators of mood.[11] The neurosteroids are efficacious modulators of $GABA_A$ receptors that act at specific binding sites to either enhance (3α-OH

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neurosteroids) or inhibit (3β-OH and 3β-sulfated neurosteroids) GABA-activated currents.[12,13] The 3α-OH neurosteroid alphaxalone (Althesin™) is an effective i.v. anaesthetic that was used clinically from 1970 to 1984; it was withdrawn from clinical use because the excipient used to solubilise it, cremophor, produced infrequent but serious anaphylactoid reactions.[14] A neurosteroid anaesthetic has never been clinically reintroduced, in part because propofol has occupied its clinical niche. Surprisingly, neurosteroid anaesthetics had not been previously evaluated in AIDN studies. Two papers by Atluri and colleagues[15] and Tesic and colleagues[16] published in the *British Journal of Anaesthesia* have addressed the effects of neurosteroids on AIDN, making several important observations that could help to unravel the Gordian knots of mechanism and prevention of AIDN.

In a 2018 paper, Atluri and colleagues[15] reported that the 3β-OH neurosteroid, (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile, induced anaesthesia/sedation comparable with that of ketamine in 7-day-old neonatal rats but did not produce the neuroapoptosis or persistent impaired spatial learning observed with ketamine. This was an intriguing result because it showed that a 3β-OH neurosteroid that neither enhances $GABA_A$ receptor currents nor inhibits NMDA receptor currents produces anaesthesia, suggesting a novel anaesthetic mechanism. The anaesthetic effect was attributed to 3β-OH blockade of T-type calcium channels, which is further supported by recent studies reported in this issue of the *British Journal of Anaesthesia* performed in mice with targeted deletion of the Cav3.1 T-type calcium channel subtype.[17]. The results also suggested that an anaesthetic without a GABAergic or NMDA blocking mechanism of action might be free of associated AIDN. An editorial accompanying the paper focused on the many practical obstacles for the development of 3β-OH as a safe and 'non-neurotoxic' anaesthetic.[18] Perhaps more germane to the question of AIDN is the absence of an important control in the study. The comparator to 3β-OH was an equi-effective dose of a non-GABAergic anaesthetic, ketamine, rather than a GABAergic neurosteroid anaesthetic. This left unaddressed the important question of whether 3β-OH is non-neurotoxic because it lacks a GABAergic effect or if neurosteroids as a class do not produce substantial AIDN.

This issue was addressed in a recent paper by Tesic and colleagues[16] comparing the anaesthetic and neurotoxic effects of two neurosteroids, alphaxalone and CDNC24, with the GABAergic anaesthetic propofol in neonatal rats. Alphaxalone and CDNC24 both activate $GABA_A$ receptors, but only alphaxalone blocks T-type Ca^{2+} channels. Both of these neurosteroids and propofol produced anaesthesia/sedation in neonatal rats. However, neither of the neurosteroids produced neuroapoptosis whereas propofol did. These results indicate that neurosteroids as a class do not produce neuroapoptosis in neonatal rats regardless of whether they activate $GABA_A$ receptors.

There are several mechanisms that could explain the observation that neurosteroids are anaesthetics but do not produce AIDN. The authors propose that neurosteroids are less efficacious activators of inhibitory synaptic currents than propofol or other GABAergic anaesthetics, allowing them to produce anaesthesia but not to achieve higher neurotoxic levels of activity. This idea is based on their data comparing the effects of neurosteroids and propofol on synaptic $GABA_A$ currents in brain slices. Alphaxalone, CDNC24, and propofol all increased the duration of spontaneous inhibitory postsynaptic currents, which is a direct effect on $GABA_A$ receptors. However, the two neurosteroids, but not propofol, also reduced the frequency of spontaneous inhibitory postsynaptic currents, a presynaptic effect likely mediated by neurosteroid action on voltage-gated Ca^{2+} channels or the synaptic release machinery. The authors proposed that the presynaptic effects of neurosteroids limit the total inhibition produced at GABAergic synapses, thus preventing neuroapoptosis. Although plausible, this hypothesis requires that the inhibitory synaptic activity required to produce anaesthesia is less than is required to produce neuroapoptosis, since the presynaptic effects of neurosteroids would otherwise prevent them from being anaesthetic. This explanation also requires that the concentrations of neurosteroid needed to inhibit presynaptic activity are higher than the concentrations that produce $GABA_A$ receptor activation. Implicit to this proposed mechanism is that there is a distinct presynaptic target that mitigates neurotoxicity and a $GABA_A$ receptor target that mediates anaesthesia and neurotoxicity. Thus, neurosteroids would be both neurotoxic anaesthetic agents and neuroprotective agents. In principle, these effects could be pharmacologically separated as they are mediated by different target sites and proteins.

I suggest two additional mechanisms that could plausibly explain the results. The first is that neurosteroids and propofol both produce anaesthesia by activating $GABA_A$ receptors, but that neurosteroids also interact with a separate target that results in neuroprotection. The presynaptic target proposed by Tesic and colleagues[16] is actually a 'special case' of this explanation. A second alternative explanation is that propofol and other anaesthetics, but not neurosteroids, interact with an unknown (non-$GABA_A$ receptor) target that mediates neurotoxicity. The three proposed mechanisms could, in principle, be experimentally distinguished by determining whether co-administration of a neurosteroid with neurotoxic anaesthetics (e.g. propofol or ketamine) reduces injury. If a neurosteroid protects from propofol, but not ketamine, this would support the proposed presynaptic neuroprotective target. If a neurosteroid protects against both propofol and ketamine, this would support a novel neuroprotective target for neurosteroids. Finally, if a neurosteroid protects against neither propofol nor ketamine it would suggest that neurosteroids are anaesthetics that simply lack neurotoxicity. Distinguishing whether neurosteroids are neuroprotectant agents or simply non-neurotoxic anaesthetics could have substantial implications for how a future clinical neurosteroid anaesthetic might be used. If neurosteroids prove to be anaesthetics with neuroprotective activity, they could ultimately be used either in combination with currently used anaesthetics that induce neurotoxicity or as a sole anaesthetising agent.

Although all three of the proposed mechanisms are plausible and testable, the idea that neurosteroids have neuroprotective effects independent of their anaesthetic effect merits additional attention. First, there is a substantial body of evidence showing that 3α-OH neurosteroids prevent apoptosis[19] and protect against nervous system injury from a number of non-anaesthetic insults.[20] Further, neurosteroids are known to bind to and modulate a number of proteins involved in cellular protection, including the progesterone receptor, orphan nuclear receptors LXR and PXR, and mitochondrial voltage-dependent anion channel (VDAC) proteins. Recent data have shown that ceramides trigger mitochondrial apoptosis by binding to a critical glutamate residue on VDAC2.[21] Neurosteroids bind to this same residue[22] suggesting they may act as competitive inhibitors, preventing a common signalling pathway for mitochondrial apoptosis.

Although a neurosteroid anaesthetic that does not cause AIDN need not have a non-GABAergic mechanism, the idea of T-type calcium channel block as a mechanism of steroid anaesthesia is of scientific interest. There are, however, some caveats in considering this result. Neurosteroids are synthesised from cholesterol in brain and 3α-OH, 3-keto, and 3β-OH neurosteroids can be enzymatically interconverted. Indeed, the anaesthetic effects of neurosteroids were discovered by Selye[23] who observed that progesterone administration causes the slow onset of anaesthesia, preferentially in female animals. Higher concentrations of the enzyme (3α-hydroxysteroid dehydrogenase) that produces 3α-OH neurosteroids are found in females than in males, and it was subsequently shown that progesterone is metabolised to a 3α-OH neurosteroid (allopregnanolone) that is responsible for the anaesthetic effect. It is also possible that 3β-OH undergoes a similar *in vivo* conversion to a 3α-OH neurosteroid that activates $GABA_A$ receptors. The 3α- and 3β-hydroxysteroid dehydrogenases in brain both work bi-directionally and could catalyse this interconversion[11] and the slow onset of 3β-OH anaesthesia reported by Atluri and colleagues[15] suggests this possibility. It should also be remembered that 3β-OH neurosteroids inhibit $GABA_A$ receptor currents. Since $GABA_A$ chloride currents can be excitatory rather than inhibitory during nervous system development, 3β-OH neurosteroids might produce an anaesthetic effect by inhibiting excitatory $GABA_A$ synaptic currents in neonatal animals. Since this would not occur in adulthood, examining 3β-OH anaesthesia in adult animals might also be instructive. Finally, it is noteworthy that the 3β-OH anaesthetic described by Alturi and colleagues[15] has a very high therapeutic index. This is unlikely to be the result of its putative non-GABAergic mechanism; neurosteroids have long been known to have higher therapeutic indices than other general anaesthetics because of their reduced cardiorespiratory depression.[14] Indeed, a reformulation of alphaxalone in sulfobutyl-cyclodextrin (Captisol™), like 3β-OH, has an unmeasurably high therapeutic index.[24]

While there is clearly much to learn about mechanisms of neurosteroid anaesthesia and neuroprotection, the two studies by Atluri and colleagues[15] and Tesic and colleagues[16] open a new door in AIDN investigation with substantial clinical implications. Anaesthetic neurosteroids, regardless of anaesthetic target, appear to produce less neuroapoptosis than do comparator anaesthetics. This result needs to be confirmed, preferably in a non-human primate model where more reliable dosing can be performed and physiologic monitoring and behavioural testing can be more thoroughly evaluated. Alphaxalone has a long record as an effective and safe anaesthetic in infants and adults[14]; it was previously withdrawn from clinical use because the excipient used in its formulation produced adverse effects, not because the compound itself was toxic. It has now been reformulated and shown to be safe and efficacious in humans.[25] Thus, alphaxalone could circumvent many of the very real obstacles to drug development cited by Vutskits and Sneyd.[18] If its lack of neurotoxicity can be validated in primates, alphaxalone presents a clear pathway to a neurosteroid anaesthetic that could be used to test for long-term neurotoxic effects of neonatal administration in humans and could be rapidly developed as a therapeutic agent. The endogenous neurosteroid allopregnanolone offers an alternative pathway to rapid human testing. Allopregnanolone is an antidepressant at sub-anaesthetic doses, and is already approved by the FDA as an i.v. infusion (Brexanolone™) for treatment of postpartum depression.[26]

There have been many disappointments in the effort to develop an anaesthetic that does not produce AIDN in animal models and could be used either experimentally to probe or therapeutically to prevent neurotoxicity in human neonates. Neurosteroids are promising new candidates in this field. They are known to be safe anaesthetics with a high therapeutic index, they are already in clinical development or use, and their well-known neuroprotective effects offer promise that 'this one could be the charm'!

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Declarations of interest

The author declares that he has no conflicts of interest.

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Predictive coding as a model of sensory disconnection: relevance to anaesthetic mechanisms

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A better understanding of the mechanisms through which we perceive our sensory environment is vital for anaesthesiology and consciousness science. Through a pragmatic approach based on tracking afferent signals, we have gradually understood how sensory stimuli are processed from the peripheral sensor, through the peripheral nervous system, into the spinal cord, thalamus, and cerebral cortex. This sensibly heralded the classical, and still dominant, view of sensory processing that focuses on feedforward transmission of sensory information to generate representations of the world around us. Here, perception relies heavily on external inputs driving neural representations of basic stimulus features in lower-order areas of the nervous system. These representations are subsequently elaborated on in successive processing stages, resulting in increasingly abstract representations in higher order cortical regions. Although there is considerable evidence to support this model, it fails to explain many phenomena such as similar physical stimuli producing alternate conscious experiences or illusions,[1]