BJA

British Journal of Anaesthesia, ■ (■): 1–11 (2018)

doi: 10.1016/j.bja.2017.12.039
Advance Access Publication Date: xxx
Laboratory Investigation

LABORATORY INVESTIGATION

A neurosteroid analogue with T-type calcium channel blocking properties is an effective hypnotic, but is not harmful to neonatal rat brain

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Abstract

Background: More than 4 million children are exposed annually to sedatives and general anaesthetics (GAs) in the USA alone. Recent data suggest that common GAs can be detrimental to brain development causing neurodegeneration and long-term cognitive impairments. Challenged by a recent US Food and Drug Administration (FDA) warning about potentially neurotoxic effects of GAs in children, there is an urgent need to develop safer GAs.

Methods: Postnatal Day 7 (P7) rat pups of both sexes were exposed to six (repeated every 2 h) injections of equipotent hypnotic doses of ketamine or the neuroactive steroid (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile (3β-OH) for 12 h. Loss of righting reflex was used to assess hypnotic properties and therapeutic index; quantitative caspase-3 immunohistochemistry was used to assess developmental neuroapoptosis; patch-clamp recordings in acute brain slices were used to assess the effects of 3β-OH on neuronal excitability and synaptic transmission. Cognitive abilities of rats exposed to ketamine, 3β-OH, or vehicle at P7 were assessed in young adulthood using the radial arm maze.

Results: The neuroactive steroid 3β-OH has a therapeutic index similar to ketamine, a commonly used clinical GA. We report that 3β-OH is safe and, unlike ketamine, does not cause neuroapoptosis or impair cognitive development when administered to P7 rat pups. Interestingly, 3β-OH blocks T-type calcium channels and presynaptically dampens synaptic transmission at hypnotically-relevant brain concentrations, but it lacks a direct effect on γ-aminobutyric acid A or glutamate-gated ion channels.

Editorial decision: January 2, 2018; **Accepted:** January 2, 2018

Conclusions: The neurosteroid 3β-OH is a relatively safe hypnotic that warrants further consideration for paediatric anaesthesia.

Keywords: calcium channels; developmental neurotoxicity; neurosteroid

Editor's key points

- Current general anaesthetics cause developmental neurotoxicity in animal models and possibly humans, creating a need for novel agents devoid of this effect.
- A neuroactive steroid (3β-OH) was shown to possess hypnotic potency without causing neuroapoptosis in neonatal rats or delayed neurocognitive deficits.
- Mechanistic investigations showed that 3β-OH blocks T-type Ca^{2+} channels and presynaptic transmitter release without affecting major postsynaptic ligand-gated ion channels.
- This provides a promising lead for development of a novel intravenous anaesthetic without developmental neurotoxic effects.

Current research evidence suggests that early exposure to clinically-used general anaesthetics (GAs) can disturb normal brain development leading to permanent cognitive and behavioural impairments in rodents,[1–4] monkeys,[5–9] and possibly in humans as well.[10–14] Currently used GAs are known to modulate two main neurotransmitter systems in the developing brain—γ-aminobutyric acid (GABA) and *N*-methyl-D-aspartate (NMDA)—which prompted us to propose that GAs with a different cellular mechanism of action might be safer and more promising alternatives. One such alternative is a class of drugs that selectively targets T-type voltage-gated calcium channels (T-channels), known to control neuronal excitability and synaptic transmission.[15] Of particular interest for our study is the neuroactive steroid, (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile (3β-OH), a potent analgesic and voltage-dependent blocker of neuronal T-currents with minimal effect on voltage-gated Na^+ and K^+ currents, N-type and L-type Ca^{2+} currents,[16,17] or recombinant $GABA_A$ and NMDA-mediated currents.[18]

Using a rat pup model, we demonstrate that 3β-OH effectively blocks T-channel-dependent excitability in thalamocortical and subicular neurones, and dampens α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-mediated excitatory transmission acting presynaptically. Furthermore, when compared with ketamine, a commonly used clinical GA, 3β-OH, is an effective hypnotic that does not cause developmental neuroapoptosis or impair cognitive development even during prolonged administration at the peak of synaptogenesis.

Methods

Animals

Most experiments were performed with postnatal day 7 rat pups (P7) (Sprague–Dawley, Envigo, Indianapolis, IN, USA), which is the peak of synaptogenesis and vulnerability to anaesthesia-induced developmental neurotoxicity.[19] Pups were housed with their mother and maintained on a 12-h light–dark cycle at a constant temperature of 21(2)°C. For radial arm maze behavioural studies, P45–P70 rats were used. For electrophysiology recordings, rat pups aged P7–P9 were used, except for studies on thalamic neurones, which used P7–P15 rat pups. Animals were housed within accredited animal facilities according to protocols approved by the University of Colorado Anschutz Medical Campus. All animals had *ad libitum* access to food and water. Treatment of rats adhered to the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimise animal suffering and to use only the number of animals necessary to produce reliable data. All experiments were approved by the Animal Use and Care Committees at the University of Colorado, the Office of Laboratory Animal Resources, Aurora, CO, USA and the Animal Use and Care Committees of the University of Virginia, Charlottesville, VA, USA. Immediately after administration of anaesthesia, pups were reunited with their mothers and allowed to nurse. Details of specific experimental procedures are provided in Supplementary material.

Results

3β-OH is an effective hypnotic

The hypnotic properties of the neuroactive steroid 3β-OH (inset in Fig. 1A) and ketamine were assessed using loss of righting reflex (LORR) in P7 rat pups injected with either agent at doses from 1 to 80 mg kg^{-1} intraperitoneally (i.p.). Since the vehicles were saline for ketamine and 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin) for 3β-OH, we included a third experimental group, ketamine+β-cyclodextrin. Rats in each group received only one dose. Neither vehicle, 15% β-cyclodextrin or saline, caused LORR (data not shown). However, 3β-OH, ketamine (KET), and ketamine+β-cyclodextrin (KET+CYCLO) caused dose-dependent shortening of the time to LORR (Fig. 1A). Data are provided as mean (SEM). The estimated ED_{50} for LORR was 3.2 (0.1) mg kg^{-1} with 3β-OH, 3.5 (0.2) mg kg^{-1} with KET and 4.1 (0.4) mg kg^{-1} with KET+CYCLO (Fig. 1B).

The estimated ED_{50} based on the duration of LORR that was obtained when either agent was injected at doses from 1 to 140 mg kg^{-1} i.p. was 39 (4) mg kg^{-1} for 3β-OH, 67 (2) mg kg^{-1} for ketamine, and 64 (2) for ketamine+β-cyclodextrin (Fig. 1C). The calculated LD_{50} for these cohorts yielded values of 63 (4) mg kg^{-1} for 3β-OH, 97 (0) mg kg^{-1} for ketamine and 95 (5) for ketamine+β-cyclodextrin (ketamine and 3β-OH LD curves included in the inset, Fig. 1D) with corresponding therapeutic indices of ~20 and ~23 for 3β-OH and ketamine+β-cyclodextrin, respectively.

3β-OH, unlike ketamine, does not cause developmental neuroapoptosis

Repeated ketamine administration for 12 h (every 2 h for a total of six doses) at 20 or 40 mg kg^{-1} induces significant