BJA

British Journal of Anaesthesia, ■ (■): 1–16 (2018)

doi: 10.1016/j.bja.2017.12.033
Advance Access Publication Date: xxx
Laboratory Investigation

LABORATORY INVESTIGATION

Inhibition of RhoA reduces propofol-mediated growth cone collapse, axonal transport impairment, loss of synaptic connectivity, and behavioural deficits

M. L. Pearn[1,2,10], J. M. Schilling[1,2,10], M. Jian[1,2,5], J. Egawa[1,2], C. Wu[3], C. D. Mandyam[1,2], M. J. Fannon-Pavlich[1,2], U. Nguyen[1,2], J. Bertoglio[6], M. Kodama[1,2,4,7], S. K. Mahata[4], C. DerMardirossian[8,9], B. P. Lemkuil[2], R. Han[5], W. C. Mobley[3], H. H. Patel[1,2], P. M. Patel[1,2] and B. P. Head[1,2,*]

[1]Veterans Affairs San Diego Healthcare System, UCSD, San Diego CA, USA, [2]Department of Anesthesiology, UCSD, San Diego, CA, USA, [3]Department of Neurosciences, UCSD, San Diego, CA, USA, [4]Metabolic Physiology and Ultrastructural Biology Laboratory, UCSD, San Diego CA, USA, [5]Department of Anesthesiology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China, [6]INSERM U749, Institut Gustave Roussy, Universite Paris-sud, Paris, France, [7]Department of Anesthesiology, National Defense Medical College, Tokorozawa, Saitama, Japan, [8]Department of Immunology and Microbial Sciences, TSRI, La Jolla, CA, USA and [9]Department of Cell and Molecular Biology, TSRI, La Jolla, CA, USA

*Corresponding author. E-mail: bhead@ucsd.edu.

[10] Pearn and Schilling share equal first authorship.

Abstract

Background: Exposure of the developing brain to propofol results in cognitive deficits. Recent data suggest that inhibition of neuronal apoptosis does not prevent cognitive defects, suggesting mechanisms other than neuronal apoptosis play a role in anaesthetic neurotoxicity. Proper neuronal growth during development is dependent upon growth cone morphology and axonal transport. Propofol modulates actin dynamics in developing neurones, causes RhoA-dependent depolymerisation of actin, and reduces dendritic spines and synapses. We hypothesised that RhoA inhibition prevents synaptic loss and subsequent cognitive deficits. The present study tested whether RhoA inhibition with the botulinum toxin C3 (TAT-C3) prevents propofol-induced synapse and neurite loss, and preserves cognitive function.
Methods: RhoA activation, growth cone morphology, and axonal transport were measured in neonatal rat neurones (5–7 days *in vitro*) exposed to propofol. Synapse counts (electron microscopy), dendritic arborisation (Golgi–Cox), and network connectivity were measured in mice (age 28 days) previously exposed to propofol at postnatal day 5–7. Memory was assessed in adult mice (age 3 months) previously exposed to propofol at postnatal day 5–7.
Results: Propofol increased RhoA activation, collapsed growth cones, and impaired retrograde axonal transport of quantum dot-labelled brain-derived neurotrophic factor, all of which were prevented with TAT-C3. Adult mice previously treated with propofol had decreased numbers of total hippocampal synapses and presynaptic vesicles, reduced hippocampal dendritic arborisation, and infrapyramidal mossy fibres. These mice also exhibited decreased hippocampal-

Editorial decision: December 26, 2017; **Accepted:** December 26, 2017

dependent contextual fear memory recall. All anatomical and behavioural changes were prevented with TAT-C3 pretreatment.

Conclusion: Inhibition of RhoA prevents propofol-mediated hippocampal neurotoxicity and associated cognitive deficits.

Keywords: axonal transport; growth cone; hippocampus; infrapyramidal; synapses

Editor's key points

- In previous studies, propofol modulates actin dynamics in developing neurones via RhoA-dependent depolymerisation of actin.
- Combined *in vitro* and *in vivo* experiments in mice showed that RhoA inhibition prevented propofol-induced synapse and neurite loss and preserved cognitive function.
- RhoA activation is a potential target for prevention propofol-induced neurotoxicity.

Anaesthetic exposure during the period of synaptogenesis in the developing brain causes widespread neurodegeneration and long-term cognitive deficits.[1–7] The spectrum of anaesthetic neurotoxicity in rodent models includes apoptosis of neurones, glia and oligodendroglia, aberrant cell cycle entry, dendritic spine and synapse loss, remodelling of the actin cytoskeleton in glia, abnormalities of mitochondrial fission, fusion, and function, and epigenetic changes that might reduce neuronal plasticity. Moreover, anaesthetic neurotoxicity in the form of apoptosis occurs not only in rodents, but also in subhuman primates. In all experimental animal models, anaesthetic-induced cognitive dysfunction appears long lasting (i.e. seen a year after anaesthesia exposure in nonhuman primates) and hippocampal in nature (i.e. affecting learning and memory). Anaesthetic neurotoxicity is a robust finding demonstrated by a number of laboratories, in a number of species of experimental animals, and with widely varying experimental protocols.

Many studies investigating anaesthetic-mediated toxicity have focused on neuronal apoptosis.[1–4,6,8–17] A number of lines of evidence, however, indicate that preventing apoptotic cell death does not reliably ameliorate anaesthetic-induced cognitive deficits.[5,18] The extent of apoptosis has been estimated to be approximately 1–2% of neurones in the cortex[19]; however, a persistent reduction in neurone number has not been reported. By contrast, there is a 50% synapse loss immediately after anaesthetic exposure in the hippocampus, and a reduction in synapse number of 10% occurs 3 months after exposure.[7] Furthermore, neuronal apoptosis occurs to a similar extent in male and female rodent pups.[20] We have shown that TAT-Pep5, a peptide that inhibits $p75^{NTR}$ mediated RhoA activation,[11] prevents isoflurane-induced apoptosis.[12] However, TAT-Pep5 administration does not ameliorate cognitive deficits.[21] Collectively, these data demonstrate that factors other than apoptosis also contribute to anaesthetic neurotoxicity and anaesthetic-induced cognitive dysfunction.

Cognitive capacity is contingent upon proper neuronal network development, which depends on proper neuronal migration, synapse formation, and network connectivity during brain development.[22–25] Neurite extension and migration is facilitated by growth cone advancement towards appropriate targets in order to establish functional circuits.[23,25] This neuronal path finding relies upon actin-dependent events, which include growth cone motility and axonal transport of neurotrophins [i.e. nerve growth factor and brain-derived neurotrophic factor (BDNF)] from nerve terminals back to the cell body and *vice versa*.[22–25] Disruption of growth cone morphology (via actin dysregulation) or impaired axonal transport leads to aberrant connectivity and impaired cognition.[23,26–30]

Actin dynamics are regulated by Rho GTPases (RhoA, Rac1, and Cdc42), small G proteins that activate/deactivate downstream effector proteins.[23,26,31] RhoA activation results in actin stress fibre formation, growth cone collapse, and impaired axonal transport; Rac1 and Cdc42 activation facilitates lamellipodia and filopodia formation, growth cone protrusion, and axonal transport.[23,27,31] The timing and balance of Rho GTPase activation is critical and necessary for proper neuronal targeting and synapse formation during the critical periods of network formation.[23,32] Dysfunctional Rho GTPase signalling results in altered actin dynamics and loss of or aberrant neuronal connectivity and cognitive dysfunction.[23,26,27,31,33,34]

We have shown that anaesthetic exposure of neonatal neurones alters Rho GTPase activity and neurite actin dynamics.[11–13] Given that regulation of actin dynamics plays an important role in neuronal pathfinding, circuit formation, and brain development,[23,26,27,31,33,34] and that propofol alters Rho GTPase signalling,[11–13] we tested the hypothesis that propofol causes neurotoxicity through increased RhoA activation, actin dysregulation, growth cone collapse, impaired axonal transport, and altered neuronal connectivity, all of which lead to cognitive deficits.

Methods

Preparation of neuronal cell cultures

All studies performed on animals were approved by Veteran Affairs San Diego Institutional Animal Care and Use Committee (San Diego, CA, USA) and conform to the guidelines of Public Health Service Policy on Human Care and Use of Laboratory Animals. Embryonic rat neurones (Sprague Dawley; Harlan Laboratories, Inc., Indianapolis, IN, USA) were isolated as described.[35] In brief, hippocampal neurones isolated from rat pups at embryonic age 16–18 were plated on poly-L-lysine coated coverslips in culture plates or within microfluidic chambers (Xona Microfluidics, Temecula, CA, USA). Axons from hippocampal neurones entered the microfluidic chamber microgroove at 3 days *in vitro* (DIV) and reached the distal axon compartments between 5–7 DIV. During neurone culture maintenance, half the media was removed and replaced with fresh maintenance media every 24–48 h.

Anaesthetic neurotoxicity model *in vitro*

Primary neuronal cultures (DIV 5–7) were placed in an incubator and exposed to 3 μM propofol (2,6-diisopropylphenol;