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Supplement Article

SUPPLEMENT ARTICLE

Inhibition of p75 neurotrophin receptor does not rescue cognitive impairment in adulthood after isoflurane exposure in neonatal mice

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Abstract

Background: Isoflurane is widely used for anaesthesia in humans. Isoflurane exposure of rodents prior to post-natal day 7 (PND7) leads to widespread neurodegeneration in laboratory animals. Previous data from our laboratory suggest an attenuation of apoptosis with the p75 neurotrophin receptor (p75NTR) inhibitor TAT-Pep5. We hypothesized that isoflurane toxicity leads to behavioural and cognitive abnormalities and can be rescued with pre-anaesthesia administration of TAT-Pep5.
Methods: Neonatal mouse pups were pretreated with either TAT-Pep5 (25 μl, 10 μM i.p.) or a scrambled control peptide (TAT-ctrl; 25 μl, 10 μM i.p.) prior to isoflurane exposure (1.4%; 4 h) or control ($n = 15$–26/group). Three to 5 months after exposure, behavioural testing and endpoint assays [brain volume (stereology) and immunoblotting] were performed.
Results: No significant difference was observed in open field, T-maze, balance beam or wire-hanging testing. The Barnes maze revealed a significant effect of isoflurane ($P = 0.019$) in errors to find the escape tunnel during the day 5 probe trial, a finding indicative of impaired short-term spatial memory. No difference was found for brain volumes or protein expression. TAT-Pep5 treatment did not reverse the effects of isoflurane on neurocognitive behaviour.
Conclusion: A single isoflurane exposure to early post-natal mice caused a hippocampal-dependent memory deficit that was not prevented by pre-administration of TAT-Pep5, although TAT-Pep5, an inhibitor of p75NTR, has been shown to reduce isoflurane-induced apoptosis. These findings suggest that neuronal apoptosis is not requisite for the development of cognitive deficits in the adults attendant with neonatal anaesthetic exposure.

Key words: behaviour; isoflurane; mouse; neurotoxicity; pharmacology

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Editor's key points

- Exposure of neonatal mammals to general anaesthetics can produce widespread neurodegeneration, but its contribution to subsequent cognitive dysfunction is unclear.
- Treatment of neonatal male mice with an inhibitor that can prevent neuronal death did not prevent delayed cognitive dysfunction following exposure to isoflurane.
- Isoflurane-induced cognitive dysfunction can be dissociated from its apoptotic effects, which suggests that other mechanisms are involved.

Isoflurane is a volatile agent that is widely used for anaesthesia in humans. Recent experimental studies indicate that exposure to isoflurane and other anaesthetics such as midazolam, nitrous oxide, sevoflurane, propofol, thiopental, and ketamine during post-natal day 7 (PND 7) leads to widespread neurotoxicity.[1–2] This neurotoxicity appears to be limited to this period of development since isoflurane does not produce neurotoxic effects at PND 15, yet does alter synaptic plasticity that persists up to 4 weeks after exposure[3].

It is postulated that neonatal exposure to anaesthetics results in neurocognitive and behavioural abnormalities during adolescence and adulthood.[2] Previous data from our laboratory show apoptosis in developing primary neurones and in the hippocampus from neonatal mice (PND 5–7) upon exposure to isoflurane.[4] This injury was mediated by preferential signalling of proBDNF via p75 neurotrophin receptor (p75NTR). Importantly, proBDNF–p75NTR signalling also plays a key role in propofol-induced neuronal degeneration.[5] Neurodegeneration induced by isoflurane and propofol was almost completely prevented by administration of the p75NTR inhibitor TAT-Pep5. Moreover, neurones from p75NTR knockout mice were not vulnerable to propofol neurotoxicity. Expression of p75NTR in neurones from p75NTR$^{-/-}$ mice recapitulated vulnerability to propofol toxicity.[5] These data suggested that a common mechanism of toxicity, which links proBDNF–p75NTR signalling to downstream RhoA activation and actin depolymerization, might be relevant to both volatile and i.v. anaesthetic developmental neurotoxicity.[6]

Given the efficacy of p75NTR inhibition in preventing anaesthetic-induced neurodegeneration, we hypothesized that isoflurane-mediated behavioural and cognitive abnormalities in mice during adulthood could be ameliorated with prophylactic administration of TAT-Pep5 prior to isoflurane exposure. To address this question we investigated the effects of a single isoflurane exposure at PND 5–7 on cognitive and behavioural function in C57Bl/6J mice 3–5 months after exposure.[7] We utilized measures of general behaviour to complement the Barnes maze platform test to assess learning and memory in the context of anaesthetic neurotoxicity.[8–14]

Methods

All studies performed on animals were approved by the Veterans Affairs San Diego Institutional Animal Care and Use Committee (IACUC) and conform to relevant National Institutes of Health guidelines. Mice were handled and habituated as described.[12]

Mice

Male and female C57Bl/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and bred in a monogamous breeding scheme to produce offspring. On a daily basis, the presence of new offspring was assessed and the gender of the pups determined.[13] On the day of the experiment, mouse pups received a toe tattoo for future identification and were randomized (by use of a random number generator) to one of four groups. PND 5–7 male pups were exposed to either isoflurane (1.4%; 4 h continuous exposure) in a 40% oxygen/air mix with a 30-min pretreatment with either a p75NTR inhibitor (TAT-Pep5; 25 μL of 10 μM, Calbiochem) or corresponding scrambled control peptide (TAT-ctrl; 25 μL of 10 μM, Calbiochem) administered i.p., or a 40% oxygen/air mix alone with either TAT-Pep5 or TAT-ctrl ($n = 16–27$/group). The temperature in the incubator was maintained at 37 °C. Behavioural testing took place at 3–5 months of age by observers without knowledge of the group allocation. Upon conclusion of behavioural testing, group allocation, which was kept by an individual not associated with the study, was revealed to the group of investigators who had conceptualized and conducted the study.

Open field activity test

Open field activity is a useful and simple test first described by Hall[14] that assesses basic activity and general behaviour/anxiety. Locomotion was recorded and analysed by computerized video-tracking system software (XT 7.1, Noldus, Wageningen, The Netherlands) software. Animals were habituated to the testing room, then spontaneous locomotion was assessed in a white Plexiglas open field box (41 × 41 × 34 cm) for 10 min. Recorded parameters were velocity (cm sec^{-1}), time spent in the centre of the arena (represented by 50% of the total arena; sec), and zone transitions.

Wire-hanging test

The wire-hanging test measures the ability of mice to hang on a metal wire.[15] The metal wire is elevated 40 cm above a soft surface to prevent physical trauma to the mice. Latency to fall was timed and the test was repeated three times with an intertrial interval of 30 s.

Balance beam test

In the beam-walking test, mice traverse an elevated, narrow beam to reach a platform.[16] The protocol measures foot slips while crossing the beam.

Continuous alternating T-maze (T-CAT) test

The continuous alternating T-maze test was used to assess cognitive ability; this enclosed apparatus is in the form of a T placed horizontally. Mice are started from the base of the T and allowed to choose one of the goal arms abutting the other end of the stem. Two trials are given in quick succession; on the second trial the mouse tends to choose the arm not visited before, reflecting memory of the first choice, termed 'spontaneous alternation'. We assessed this tendency in a test with 14 possible alternations as previously described .[12 17 18]

Barnes maze test

The Barnes maze (BM)[19] was designed for testing spatial learning and memory.[20] The BM is preferred over the Morris water maze to assess spatial memory in mice, taking advantage of their superior abilities to find and escape through small holes while minimizing the motor-dependent component in the task.